



Abstracts book
XLVIII Annual Meeting
Chilean Society for Biochemistry and
Molecular Biology

2025

6th - 9th October
Hotel Club, La Serena, Región de Coquimbo



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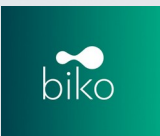
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CONFERENCE ABSTRACTS

IUBMB Opening lecture

“From Bench to Bedside: The Transformative Power of Induced Pluripotent Stem Cells.”

Ángel Raya, MD/PhD

ICREA Research Professor at the Bellvitge Biomedical Research Institute (IDIBELL); Director, Program for Clinical Translation of Regenerative Medicine in Catalonia (P-CMR[C]); Group Leader at CIBER-BBN; Full Professor at the Department of Physiology, University of Barcelona.

The revolutionary discovery of induced pluripotent stem cells (iPSCs) by Takahashi and Yamanaka is redefining the boundaries of biomedicine and offers a unique bridge between basic research and clinical application. In this talk, I will discuss how this technology is driving a new paradigm in personalized medicine, from disease modeling to innovative therapeutic strategies. iPSCs have proven to be exceptional tools for unraveling the pathogenesis of complex diseases. By generating specific cell types (such as neurons or cardiomyocytes) from patients, we have been able to recreate neurodegenerative and cardiac disorders *in vitro* with unprecedented precision. Our work has explored how these platforms allow us to dissect the genetic complexity of human diseases and the specific contributions of various cell types to disease phenotypes. These findings make it possible not only to identify molecular signatures of diseases but also to validate therapeutic targets in genetically relevant contexts. Beyond modeling human diseases, iPSCs are driving a paradigm shift in therapy development. I will discuss advances in the clinical translation of iPSC-based applications, addressing critical challenges related to scalability, safety, and functional integration. Emerging innovations, such as CRISPR-based gene editing for disease correction and organoid/3D bioprinting platforms for tissue engineering, will further expand the therapeutic potential of iPSCs. By connecting basic science and clinical innovation, iPSC technology is redefining personalized medicine. This talk will highlight our contributions to the field and outline a roadmap for turning reprogramming breakthroughs into transformative treatments.

Severo Ochoa Lecture

“CRISPR genome editing applied to research on rare diseases”

Almudena Fernández, Gema Garrido, Marta Cantero, Julia Fernández, **Lluís Montoliu**.

National Centre for Biotechnology (CNB-CSIC) and Spanish National Network for Research on Rare Diseases (CIBERER-ISCIH), Madrid, Spain

Rare diseases affect 1:2.000 newborn but there are more than 6.000 of these pathologies hence affecting millions of people: 3 millions in Spain, about 30 millions in Europe and more than 300 millions world wide. The very low incidence of these rare diseases among the population is basically the only feature they have in common. They can affect any organ or tissue in the body. Due to the scarce amount of patients for each of these pathologies often we use animal models to replicate genotypes and phenotypes observed among those patients. In particular, thanks to the CRISPR-Cas9 genome editing tools, we can now generate avatar mice, mouse models carrying patient-specific mutations, in order to reveal their direct or indirect contribution to the symptoms or consequences associated with the disease. In my lab we have been studying albinism for more than 30 years. Albinism is one of the rare genetic conditions. There are 22 types of albinism, associated with at least mutations in 21 genes. All types of albinism show visual alterations (foveal hypoplasia, abnormal connections between the retina and the visual nuclei in the brain, nystagmus and photophobia) but not all types of albinism display obvious hypopigmentation. Over the years we have generated a series of avatar mice representative of a number of albinism types, which we have phenotyped and characterized, thereby increasing our understanding of this pathology and progressively approaching the development of treatments (small molecules, gene therapy...) to fix the visual phenotype and, possibly, the hypopigmentation. In this talk I will summarize the work done by our lab and other labs in the field using mouse models of albinism.

Oswaldo Cori Lecture

“From CO₂ Fixation to Cell-Wall Deposition: Carbon Routing Across Photosynthesis and Golgi Transport.”

Ariel Orellana.

Centro de Biotecnología Vegetal, Facultad de Ciencias de la Vida, Universidad Andrés Bello,

Plants are Earth’s primary biological engine for removing CO₂ and converting it into biomass, much of which is locked into the plant cell wall. Drought and heat already constrain this process. We follow a single carbon stream—from atmospheric CO₂ to durable wall polymers—linking capture and use in one continuum. In the Atacama annual *Cistanthe longiscapa*, photosynthesis shifts with water: strong CAM under drought (nocturnal CO₂ uptake; high water-use efficiency) and a C₃-like mode under irrigation (daytime stomatal opening). Physiology, photoprotection, and diel gene expression change coherently across aridity and irrigation, positioning *Cistanthe* as a tractable model to identify molecular switches that tune carbon capture in crops.

This captured carbon flows from chloroplast triose-phosphates into sucrose—the principal product of photosynthesis and the transport currency—then into the nucleotide-sugar pools that provide the building blocks of biomass. Cellulose is synthesized at the plasma membrane, whereas hemicelluloses and pectins are assembled in the Golgi by luminal glycosyltransferases that require cytosolic donors. Nucleotide-sugar transporters (NSTs) import those donors, setting luminal supply. We identified and dissected Arabidopsis NST families showing that mutants alter polysaccharide architecture, the network compensates when one route is disrupted, and closely related transporters have context-dependent roles.

Together, these results define an integrated carbon pipeline—water-responsive capture feeding Golgi-mediated wall biosynthesis—and suggest concrete strategies to maintain or enhance plant carbon sequestration as climates warm and dry.

Funding: Fondecyt 1230859; ANID – Millennium Science Initiative Program – ICN2021_044; Mizutani Foundation for Glycoscience, Grant No 240062.

SIMPOSIUM ABSTRACTS

Simposium 1: “Molecular basis for microbial pathogenesis and immunity”

Organizers: Pablo González y Alexis Kalergis

The two faces of senescence: Aging and its Role in Bacterial Virulence Factors. Diego Ormeño¹, Mauricio Garrido¹, Christian Fernández¹ and Mónica Cáceres¹ (monicacaceresll@uchile.cl).

¹Institute of Biomedical Sciences (ICBM), Faculty of Medicine, Universidad de Chile, Santiago, Chile.

²Millennium Institute on Immunology and Immunotherapy (IMI).

Cellular senescence is a complex intracellular process with diverse biological, physiological, and pathological roles. Pathologically, its persistent activation can contribute to aging. In this context, we observed accumulation of senescent cells during human aging gingival tissue, by increased levels of γ H2A.X, 53BP1, and SAHF, along with a greater distance of H3K9me3 from the nuclear periphery and gingival senescent fibroblasts (GSF). To evaluate the removal of senescent cells through the immune system, we co-cultured young Peripheral Blood Mononuclear Cells (PBMC) with senescent cells from both young and aged donors. GSF from aged donors recruited PBMC more slowly and in fewer numbers compared to those GSF from younger donors, suggesting impaired immune surveillance with age. In a physiological context, we also identified senescent cells in proximity to dental caries in young donors. Exposure to microbial components- Lipopolysaccharide (LPS) from *Porphyromonas gingivalis*, lipoteichoic acid (LTA) or Peptidoglycan (PG) from *Bacillus subtilis*- induced either proliferation or senescence depending on concentration. These findings underscore the dual role of senescence in oral health and disease.

Deciphering the molecular mechanisms of HIV-1-induced immune activation in human microglia. Aracelly Gaete-Argel^{1,2,3*}, Catarina Ananías-Sáez^{1,2,3*}, Cecilia Rojas-Fuentes^{1,2,3}, Sebastian Giraldo-Ocampo^{1,2,3}, Daniela Jara-Araya^{1,2,3}, Tomás Hernández-Díaz^{1,2,3}, Camila Ortega-Orellana^{1,2,3}, Lucía Cortesi^{1,2,3}, Delia López-Palma^{1,2,3}, Hisashi Akiyama⁴, Suryaram Gummuluru⁴, Pablo A. González^{3,5}; Fernando Valiente-Echeverría^{1,2,3}, Ricardo Soto-Rifo^{1,2,3} (rsotorifo@uchile.cl).

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⁴Department of Microbiology, Boston University School of Medicine, Boston, MA, 02118, USA. ⁵Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile. *These authors should be considered as joint first authors.

Microglia are the main target for HIV-1 infection in the central nervous system being considered a major contributor for neuroinflammation in people living with HIV (PLWH). Still, the mechanisms leading to the induction of inflammatory mediators during HIV-1 infection in the brain remain elusive. Here, we show that HIV-1 infection in microglia is not associated with a classical antiviral response but rather to the induction of a subset of pro-inflammatory mediators including IL-6 and IL-8. Interestingly, this inflammatory response was independent from the sensing of the intron-containing RNA previously reported in macrophages and dendritic cells but resulted from the activation of a basal immune signaling dependent on the DEAD-box RNA helicase DDX3X by the HIV-1 accessory protein Nef. We also observed the DDX3X-dependent and Nef-independent transcriptional activation of the neuroprotective cytokine IL-11 as a cellular countermeasure to HIV-1-induced inflammation. Together, our results reveal the complexity of the inflammatory response of human microglia during HIV-1 infection providing unprecedented mechanistic insights for the understanding of neuroinflammation in PLWH.

Salmonella in a One Health context: a genomic approach to understanding the molecular basis of emerging Salmonella serotypes of public health importance. Andrea I Moreno-Switt (andrea.moreno@uc.cl).

Escuela de Medicina Veterinaria, Pontificia Universidad Católica de Chile

Emerging *Salmonella* serotypes threaten public health by combining antimicrobial resistance (AMR), virulence traits, and environmental persistence, requiring a One Health perspective that integrates human, animal, food, and environmental reservoirs. Whole-genome sequencing (WGS) provides the molecular resolution to dissect the genetic mechanisms underpinning their emergence. In Chile, 2,800 isolates were collected from agricultural water, foods, wild birds, and human clinical cases. Genomic characterization revealed the presence of repetitive, emerging and persistent strains (REP strains) of the predominant serotypes of *S. Infantis*, *S. Typhimurium*, *S. Newport*, and *S. Agona*. Notably, *S. Infantis* carried a multidrug-resistant lineage defined by the pESI-like megaplasmid, which encoded extended-spectrum β -lactamases, fluoroquinolone resistance determinants, and disinfectant/heavy-metal resistance genes. This plasmid also harbored toxin–antitoxin systems and virulence-associated genes that enhance plasmid stability and bacterial fitness. In addition, genomic islands and mobile genetic elements were identified as hotspots for AMR and virulence gene acquisition. Phylogenomic analysis showed that clonal *S. Infantis* lineages were disseminated across rivers, foods, and human cases, suggesting environmental and foodborne pathways for transmission. Beyond resistance, the presence of Type III secretion system effectors, fimbrial operons, and stress-response determinants highlighted molecular adaptations that may facilitate persistence in diverse hosts and environments. By integrating genomic surveillance into a One Health framework, this study reveals how plasmids, genomic islands, and virulence repertoires drive the emergence and success of *Salmonella* serotypes. Understanding these molecular mechanisms is essential for developing targeted interventions to curb AMR and reduce the public health impact of *Salmonella*.

Funding: FONDECYT 1231082, 3230796, and FDA Cooperative Agreement to Support the Joint Institute for Food Safety and Applied Nutrition (JIFSAN).

Post-transcriptional regulation of viral gene expression by host RNA-binding proteins. Marcelo López-Lastra¹ (lopezlastra@uc.cl).¹Laboratorio de Virología Molecular, Instituto Milenio de Inmunología e Inmunoterapia, Departamento de Enfermedades Infecciosas e Inmunología Pediátrica, Escuela de Medicina, Pontificia Universidad Católica de Chile, Chile.

Viruses rely on the host's protein synthesis machinery to translate their mRNAs (vRNAs). Early during infection, vRNAs and host mRNAs compete for the same translational machinery. To be successful in this task, many vRNAs utilize non-canonical strategies, such as internal ribosome entry sites (IRESs), to recruit the host translational machinery, overpowering host mRNAs. Interestingly, most viral IRESs require host RNA-binding proteins (RBPs), other than the canonical translation initiation factors, to enable and regulate their activity. Curiously, most RBPs required to regulate viral IRESs are predominantly nuclear in noninfected cells. Thus, vRNAs subvert nuclear RBPs to accomplish vRNA translation. This session will discuss examples of IRES-mediated strategies used by vRNAs. Novel findings will be presented, highlighting the relevance of host RBPs and their post-translational modification in translational control of viral gene expression.

Funding: The work was supported by grants FONDECYT 1250350 and the Iniciativa Científica Milenio (ICM), IMII (ICM-ANID, ICN2021_045).

Simposium 2: "Molecular Motion meets function: Insights from Structural Dynamics"

Organizadores: Víctor Castro-Fernández y Exequiel Medina G.

Frustrated Flexibility Shapes the Dynamics of Calmodulin Trapping. Narendar Kolimi¹, Rajen Goutam¹, M. Neal Waxham², Exequiel Medina³, [Hugo Sanabria](mailto:hsanabr@clmson.edu)^{1,4} (hsanabr@clmson.edu).

¹Department of Physics and Astronomy, Clemson University, Clemson 29634, United States. ²Department of Neurobiology and Anatomy, University of Texas Health Science Center at Houston, Houston, Texas, 77030, United States. ³Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, 8380494, Chile. ⁴ Medical Biophysics, Clemson University, Clemson, 29634, United States.

Calmodulin (CaM) is a universal calcium sensor that regulates diverse signaling pathways through its conformational flexibility. A key interaction is with Ca²⁺/calmodulin-dependent protein kinase II (CaMKII), which uniquely retains CaM after calcium levels decline, a process known as CaM trapping and central to synaptic plasticity. Despite its importance, the molecular basis of CaM trapping has remained poorly defined, particularly the role of conformational dynamics. Here, we employed single-molecule Förster resonance energy transfer (smFRET) and structural ensemble modeling to resolve the conformational landscape of CaM in its free, calcium-bound, and CaMKII-bound states. Using 11 FRET-labeled CaM variants, we show that CaM rapidly interconverts among multiple conformations, revealing hidden structural states incompatible with static models. Our results demonstrate that CaM trapping is not a rigid endpoint but a dynamic process involving a frustrated conformational landscape. These findings establish conformational dynamics as a critical determinant of CaM-mediated signaling and provide a mechanistic framework with broad implications for long-term potentiation, synaptic plasticity, and other CaM-dependent processes.

Disordered DNA-Binding Proteins: Structural Dynamics in Gene Regulation. Elias Manriquez¹, Pedro General¹, Pablo Villalobos², [Exequiel Medina](mailto:exequiel.medinago@uchile.cl)¹ (exequiel.medinago@uchile.cl).

¹Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile. ²Facultad de Ciencias, Universidad de Chile.

Eukaryotic transcription factors (TF) are crucial to regulate gene expression, and, compared to their prokaryotic counterpart, contain not only extended disordered regions -that do not fold into a stable three-dimensional structure- but also an intricate combination of disordered and folded domains, highlighting the complex (un)structure-function relationship. Among those domains, we are interested in the DNA-binding domains (DBDs) and the impact of the DNA binding and the interdomain communication on structural dynamics and function. One TF model is the human forkhead box P subfamily, which contains a canonical and highly conserved DNA-binding domain (FKH), that folds and adopts a three-dimensional swapping dimer in the absence and presence of DNA. Another TF model under our investigation is the DBD of the damage suppressor protein (DSUP) from the tardigrade *Ramazzottius varieornatus*. *This intrinsically disordered protein* protects the DNA from radiation and allows this organism to survive in extreme conditions. Labeling different positions of these proteins with Alexa488 (donor) and Alexa647 (acceptor), we employed single-molecule FRET experiments to study these DNA-binding domains, to probe their high-resolution structural dynamics in the absence and presence of DNA. Interestingly, we determined that the structural dynamics of FKH strongly depend on the quaternary structure, where the monomer is in equilibrium between a favored disordered-like and a folded state, and dimerization is an example of a folding-upon-binding process. Similar to the monomeric FKH, DSUP showed highly dynamic conformations in the absence of DNA. However, the presence of the specific DNA ligand evoked different effects on FKH and DSUP, favoring the dissociation of the dimeric FKH and therefore increasing its disordered behavior. In contrast, for DSUP, the ligand does not change the equilibrium between disordered and folded states but increases both transition rates. These results highlight first, the need to revisit the canonical vision of the forkhead's DNA-binding domain and the regulatory role of the DNA on its dimerization equilibrium, and secondly, the need to explore more in detail the structurally complex behavior of DSUP and the relation with the DNA protection role in tardigrades.

Acknowledgments: Fondecyt inicio 11200729, Fondecyt regular 1251879, Fondecyt postdoctorado 3230600, and Fondecyt EQM200202

AlphaFold2 effectively classifies the functional diversity in a protein family. Pablo Galaz-Davison (pablogalazdavison@gmail.com).
CBSM, Universidad de Talca.

AlphaFold2 (AF2) excels in predicting static folds but struggles with conformationally dynamic proteins. RfaH is a transcription factor found in enterobacteria that undergoes an α -to- β switch of an entire α -folded domain to achieve gene-specific regulation, and its fold-switching has been intensively modeled as benchmark for predictors of structural duality. Previous reports by us and others have suggested the presence of predictions of β -folded domains in the RfaH family are the result of contamination from NusG sequences, a monomorphic protein sharing the common active β -fold of RfaH and from which RfaH diverged. In this study we sought to determine whether these static folds predicted by AF2 are correct through experimental data. For this end, we predicted the structures of ~5,000 RfaH sequences, analyzed the phylogeny and genomic context of relevant sequences and experimentally determined their activity profile. Our results show that AF2 captures the diversity across RfaH orthologs, classifying them into three distinct categories: monomorphic, metamorphic, and mixed α/β . Phylogenetic analysis on these sequences agrees with this stratification by aggregating them on clades with similar structural prediction. For the monomorphic RfaH, unreported so far, it was observed that these sequences were right next to the targets they controlled, not necessitating fold-switching as a regulation mechanism. Experimental validation using reporter assays in *E. coli* reveals sequences from a monomorphic clade are constitutively active, sequences from metamorphic clades display switchable activity, while sequences from mixed α/β clades show domain instability. Crucially, through hundreds of short targeted molecular dynamics we observe that AF2 interpretation of instability gives rise to these mixed α/β RfaH. This work shows using default AF2 can be used to interpret structural variability in a protein family.

Acknowledgment: FONDECYT 3240319

The Role of Conformational Dynamics in the Allosteric Regulation of PFK/GK Enzymes of Methanogenic Archaea. Sixto M Herrera¹ (sixto.morales@ug.uchile.cl), Hugo Sanabria², Victor Castro Fernandez¹, Victoria Guixé¹.

¹Laboratorio de Bioquímica y Biología Molecular, Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile. ²Department of Physics and Astronomy, Clemson University, Clemson, South Carolina.

The bifunctional ADP-dependent phosphofructokinase/glucokinase (PFK/GK) enzymes from methanogenic archaea are activated by their product, AMP, an unusual regulatory mechanism. This product-activation loop is a conserved feature among bifunctional ADP-dependent sugar kinases in archaea. Structurally, these enzymes consist of a conserved architecture comprising a large Rossmann-like domain and a smaller lid domain, with the active site located at their interface. To address the conformational changes underlying catalysis and AMP activation, we employed two complementary methodologies: single-molecule fluorescence and molecular dynamics simulations on the PFK/GK enzymes from *Methanococcus marisaludis* and *Methanothermococcus thermolithotrophicus*. Our findings reveal that distinct conformational landscapes can give rise to the same regulatory outcome. Notably, the two enzymes differ in how substrates and AMP stabilize the catalytically relevant closed conformation. Furthermore, we found that the allosteric binding site is formed only in the closed conformation, uncovering a conformation-dependent regulatory mechanism. Our results show that distinct conformational landscapes can give rise to the same regulatory outcome. Notably, the two enzymes differ in how substrates and AMP stabilize the catalytically relevant closed conformation. Furthermore, we found that the allosteric binding site is formed only in the closed conformation, uncovering a conformation-dependent regulatory mechanism. This finding demonstrates that distinct conformational dynamics can sustain the same conserved regulatory trait, offering deeper insights into the evolutionary interplay between molecular motion and enzyme function.

Acknowledgments: Fondecyt 1231263

Simposium 3: “Basic Science and Biotechnology a tribute to Luis Burzio”

Organizer: Raúl Herrera

Learning from fruit ripening to biotech applications. Raúl Herrera (raherre@utalca.cl) and M. Alejandra Moya-León.

Instituto de Ciencias Biológicas, Universidad de Talca.

Fruit ripening is a development process modulated by several plant hormones. The right coordination of ABA, auxins and ethylene triggers physiological and biochemical changes associated with softening, color or flavor/aroma. The modification of plant cell walls takes place during ripening, resulting in the alteration of the structure and/or composition of cell wall polysaccharides. During softening, the disassembly of the cell wall structure is mediated by the orchestrated participation of a wide variety of cell wall degrading enzymes. Apart from the involvement of plant hormones, a set of transcription factors have been reported as the organizers of this multi-protein effort. Enzymes such as XTH, PG, EG, RGL, EXP are modulated in their expression and participate in the breakdown of glyco-bonds from cellulose, hemicellulose and pectins. Transcriptional factors are key modulators, not just for many cell wall degrading enzyme genes, but also for color and aroma related genes. In this sense, the synthesis of anthocyanins is regulated at transcriptional level by the triad MYB, basic helix-loop-helix (bHLH) and WD-40 repeat proteins, forming the MBW complex. It has been reported in *F. chiloensis* fruit the repression effect of *FcMYB1*, as its silencing increases the level of anthocyanins and stimulates the expression of genes of the anthocyanin biosynthetic pathway such as *CHI*, *F3H*, *DFR* and *ANS*. On the other hand, AUX/ABA modulates the expression of alcohol acyltransferases (AAT), key enzymes of ester biosynthesis. Several AAT isoforms are expressed during ripening, which could be involved in the generation of the wide diversity of esters produced by the fruit. These findings expand our understanding on the molecular basis of fruit ripening but also raise the need for further research to fully understand the process. Besides, knowledge generated opens opportunities for developing biotechnological strategies aiming for the improvement of fruit quality or increasing the production of esters.

Acknowledgement: ANID-FONDECYT 1251538.

Extreme-inspired biotechnology: bridging the gap from wet lab to Agritech industry. Denisse Bravo (denisse.bravo@unab.cl). Facultad de Odontología, Universidad Andres Bello, Santiago Chile.

Extreme ecosystems, such as Antarctica, hyper-arid deserts, volcanic regions, and subpolar zones, host highly specialized microbial communities known as extremophiles. These organisms have evolved unique adaptive mechanisms to withstand severe abiotic conditions, including very low temperatures, intense UV radiation, water scarcity, and high salinity. In recent decades, the functional characterization of extremophiles has opened new areas of development in agricultural biotechnology, particularly in the development of biological products focus to enhance crop resilience under extreme climatic events. For instance, bacterial strains isolated from Antarctic plants have shown the ability to induce cold tolerance in diverse crops through multiple protective mechanisms against abiotic stress. Similarly, microorganisms from desert soils display osmoprotective and biostimulant properties, useful for growth cultures with prolonged drought and low soil biodiversity. Such strategies permit to replace the use of synthetic chemical products with biodiversity-driven solutions, fostering more sustainable agricultural systems adapted to the new climate scenarios. Altogether, the study of extremophiles not only expands our fundamental microbiological knowledge but also provides a versatile platform for next-generation agricultural technologies aimed at strengthening food security and mitigating the impacts of climate change on crop production.

Acknowledgments: Fondecyt 1251126

The road from the wet lab to cellulosic industry. Rafael Vicuña (rvicuna@bio.puc.cl). Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

The emergence of biotechnology in the 1980s faced academic researchers with the dilemma of continuing to conduct basic research or engaging in the growing trend of industry-oriented research. Universities developed specific policies for enabling researchers to contribute to the country's productive development without hindering the pursuit of new knowledge. This presentation will showcase a specific case in which basic science was successfully combined with the development of large-scale biotechnological processes. Our group addressed the topic of lignin biodegradation. This polymer, which represents the second largest reservoir of organic carbon in nature, is closely associated with cellulose and hemicellulose in the plant cell wall. Its chemical structure is extremely complex, consisting of various phenylpropanoid units that associate with each other through various types of linkages, giving rise to an irregular, insoluble, and non-hydrolysable polymer. In the course of our work, we discovered that the enzymes that degrade lignin are multi-copper oxidases and peroxidases, which attack the polymer nonspecifically. These enzymes are produced by certain basidiomycete fungi, the only microorganisms in nature that possess ligninolytic activity. In particular, we characterized the ligninolytic system of the fungus *Ceriporiopsis subvermispota*, including the mechanism involved in the production of the hydrogen peroxide required by peroxidases. In addition to characterizing the enzymes and studying their mechanisms of action, we obtained the complete sequence of the fungal genome, which allowed us to study the structure and regulation of the expression of the genes that encode them. In parallel to these basic studies on ligninolysis carried out in the university laboratories, we developed a biotechnology program with the company Celulosa Arauco and Constitución, which was carried out entirely in industrial facilities. This program included three lines of work: a) biopulping (pretreatment of wood chips with ligninolytic fungi to facilitate their subsequent chemical pulping); b) biobleaching (treatment of cellulose pulp with enzymes to reduce the use of chlorinated reagents in the usual bleaching process); and c) treatment of effluents with ligninolytic microorganisms to reduce their organochlorine content. Biopulping was partially successful on a pilot scale, while biobleaching and effluent treatment were successful on an industrial scale.

Bridging the gap: overcoming challenges in applying transcription factor research from model systems to crop improvement in field environments

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Cátedra de Biología Celular y Molecular (FBCB-UNL).

Plants exhibit sophisticated signal transduction pathways to adapt to adverse conditions, with transcription factors (TFs) acting as crucial molecular switches. 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 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 became rare, yet successful, cases commercially 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 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 remaining complexities. Our investigations led us to hypothesize that the differential traits conferred by these transgenes might be triggered by small RNAs generated by the plant as a defense mechanism. To test this, we developed novel genetic constructs designed to produce elevated levels of small RNAs derived from the HaHB4 sequence. Transforming Arabidopsis plants with these constructs yielded amazing results: a significantly enhanced seed yield. Crucially, this beneficial phenotype was lost when the constructs were expressed in Arabidopsis mutants unable to generate small RNAs, strongly supporting our hypothesis. Although further research is needed to fully elucidate how these small molecules enhance crop desirable characteristics, these findings highlight their immense potential as biotechnological tools for crop improvement. We are currently analyzing soybean plants transformed with the same constructs, and our discussion will explore both the mechanistic and biotechnological implications of these discoveries.

Symposium 4 “New Perspectives on Cell Wall Biosynthesis and Signaling”

Organizer: Ariel Orellana

Insights into how spatial and temporal calcium signaling events separately regulate cellular morphogenesis.

Magdalena Bezanilla (magdalena.bezanilla@dartmouth.edu).

Department of Biological Sciences, Dartmouth College, NH, USA

Polarized growth drives the morphogenesis of elongated cellular structures such as axons and dendrites in neurons and root hairs and pollen tubes in plants. In plants, polarized cells exhibit a tip focused ionic calcium gradient that has a complex oscillatory behavior. Whether the spatial or the temporal characteristics of this gradient underlie a role in growth has been unclear. We discovered that autoinhibitory calcium ATPases (ACAs) redundantly contribute to polarized growth. Cells lacking ACA function have a diminished calcium gradient and significantly reduced apical secretion but exhibit normal calcium fluctuations, thereby separating the spatial and temporal characteristics of the gradient and providing a tool to investigate the contributions of each to polarized growth. Spatial regulation of calcium efflux was explained by examining ACA localization. ACA1 and ACA2 localize to the subapical plasma membrane and ACA5 to the vacuole membrane. Thus, calcium export from the cytoplasm occurs only in areas where the ACAs are localized and activated by higher calcium levels. Tip growing plant cells also require actin for growth. Actin is found near the apex of the cell and is regulated by the temporal fluctuations in calcium. As expected, since the calcium fluctuations are not altered in cells lacking ACA function, these cells also have normal actin. Furthermore, cells lacking apical actin retain a strong calcium gradient but have reduced apical secretion. Suppression of both the calcium gradient and apical actin dramatically impairs growth, supporting a model where two independent and parallel processes, the spatial calcium gradient and apical actin, promote rapid polarized growth.

Cell Wall-Associated Signaling Under Aphid Attack: GWAS and Functional Characterization of CSL Genes in Arabidopsis Defense.

Francisca Blanco-Herrera^{1,2,3} (mblanco@unab.cl), Felipe Moraga¹, Daniela Arias-G^{1,3}, Joaquín Delgado-Rioseco^{1,3}, Diego Zavala^{1,2}, Francisca-Ormeño¹, Dayan Sanhueza¹, Susana Saez-Aguayo¹, and Ariel Herrera-Vasquez¹.

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Aphids are among the most damaging agricultural pests due to their rapid asexual reproduction, dispersal capacity, and ability to transmit plant viruses. They weaken plants by withdrawing nutrients from phloem tissues, yet the molecular basis of plant resistance to phloem-feeding insects remains poorly understood. To investigate the genetic determinants of resistance in *Arabidopsis thaliana* against the specialist aphid *Brevicoryne brassicae*, we performed a genome-wide association study (GWAS) on 200 natural accessions. Resistance was assessed by aphid reproduction, revealing Kondara and Tsu-1 as the most resistant accessions, with significantly fewer offspring than Col-0. Marker-trait association identified SNPs on chromosomes 2 and 4, with candidate genes enriched in cell wall (CW) metabolism. Focusing on CSLB4, a cellulose synthase-like gene implicated in hemicellulose biosynthesis, we found that *cslb4-1* mutants exhibited enhanced resistance, with reduced aphid offspring and preference. Electrical Penetration Graphs showed increased aphid activity and phloem ingestion on *cslb4-1*, yet overall reproduction declined, indicating compromised feeding efficiency. CW analysis revealed altered hemicellulose and pectin composition, including reduced xyloglucan in the epidermis. Moreover, *cslb4-1* mutants upregulated defense-related genes such as *PDF1.2* and *RBOHD*, linking CW remodeling with jasmonic acid signaling and reactive oxygen species production. Together, these results demonstrate that changes in xyloglucan content or structure can activate compensatory defense pathways, enhancing resistance to *B. brassicae*. This study highlights the importance of CW composition in modulating phloem-feeding interactions and expands our understanding of cell wall integrity sensing as a key regulator of plant immunity.

Acknowledgment: Funding, Programa Iniciativa Científica Milenio-ICN17_022, NCN2021_010, and ANID PIA/BASAL FB0002, and Fondecyt Postdoctorado 3230451.

A Reductionist Approach to Determining the Biochemical and Biological Functions of the Complex Plant Cell Wall Pectic Polysaccharides Reveals a Structural/Functional Expansion of the Homogalacturonan Biosynthetic GAUT gene family from Cyanobacteria to Green Algae and Higher Plants. Debra Mohnen^{1,3} (dmohnen@ccrc.uga.edu), Kristen Engle McAdams^{2,3}, Clifford Okoye^{1,3}, James Leebens-Mack², Desiree Moore^{1,3}, Bennett Cobb^{1,3}, Melani Atmodjo^{1,3}.

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Pectins are the most complex glycans in the plant cell wall. They are a family of cell wall glycans and proteoglycans that contain 4-linked D-galacturonic acid (GalA). All pectins have either a homogalacturonan (HG) α -1,4-linked GalA backbone or a repeating [α -1,4-D-GalA- α -1,2-L-Rha] disaccharide repeat backbone. The major pectic glycans with an HG backbone are HG, rhamnogalacturonan II (RG-II) and the less abundant xylogalacturonans and apiogalacturonans. Rhamnogalacturonan I (RG-I) has a disaccharide GalA-Rha repeat backbone and a diversity of structures due to the variety and spacing of its galactan, arabinogalactan and arabinan side chains and degree and pattern of acetylation and methylation. The *GALACTURONOSYLTRANSFERASE* (GalAT) (*GAUT*) family encodes catalytic and Golgi-anchoring subunits involved in the synthesis of HG and heteroglycans. Structural data show that HG and RG-I and HG and RG-II are covalently connected together as complex heteroglycans in the wall, although some HG may exist as a homopolymer. Some, perhaps most, of the RG-I in the cell wall is present as an arabinogalactan proteoglycan. The talk will summarize our current understanding of the structure and biological functions of this expansive gene family (15 members in Arabidopsis) and strategies to dissect structure and function via a phylogenetic/enzymatic/molecular genetic approach.

PMEs and PMEIs in seed mucilage: A coordinated team regulating homogalacturonan methylation. Dayan Sanhueza¹, Ricardo Yusta¹, Pablo Sepúlveda-Orellana¹, Juan-Pablo Parra-Rojas¹, Ariel Orellana¹, Carlos Gaete-Eastman², Raúl Herrera², Jérôme Pelloux³, Jossip Safran³, Asier Largo Gosens¹ and Susana Saez-Aguayo¹ (susana.saez@unab.cl).

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The plant cell wall is a complex and dynamic structure mainly composed of cellulose, hemicellulose, and pectins. Homogalacturonan (HG), the most abundant pectin, is a homopolymer of galacturonic acid that can be methylesterified or not, a modification critical for regulating wall properties such as stiffness through “egg-box” structures. HG methylesterification is controlled by pectin methylesterases (PMEs) and their inhibitors (PMEIs), which are highly redundant gene families in Arabidopsis (69 PMEs and 72 PMEIs). This redundancy has hindered functional analyses. Arabidopsis seed mucilage, a pectin-rich and easily extractable extracellular matrix, provides a valuable model to dissect HG methylesterification. Through mutant screening, we identified two PMEIs (PMEI3, PMEI17) and two PMEs (PME31, PME51) required for proper mucilage structure, as their mutants displayed altered ruthenium red staining. Chemical analyses of *pmei* mutants indicated that PMEI3 and PMEI17 fine-tune HG methylation. Remarkably, *pme31* and *pme51* mutants showed a distinctive chemotype with ~40% higher rhamnose and GalA content, while methanol levels remained unchanged. In vitro assays with heterologously expressed PME31, PME51, and PME58 confirmed PME activity, and bioinformatic analyses demonstrated that PMEI3 and PMEI17 inhibit PME51 and PME58. Moreover, triple *pme31pme51pme58* mutants revealed phenotypes unrelated to individual PME mutations, highlighting the existence of compensatory mechanisms that stabilize mucilage composition despite altered methylesterification. Overall, our study uncovers a coordinated set of PMEs and PMEIs regulating HG methylation in seed mucilage, providing new insights into how redundancy and inhibition ensure robustness in pectin modification.

Symposium 5: “Organelle Communication as a Platform Signaling in Chronic Diseases”

Organizers: Valentina Parra y Julio César Cárdenas.

Mfn2 Actions in Membrane Contact Sites. [María Isabel Hernández-Alvarez \(mihernandez@ub.edu\)](mailto:mihernandez@ub.edu)^{1,2,3}.

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Mitofusin 2 (Mfn2) is a key GTPase located in the outer mitochondrial membrane, best known for its role in mitochondrial fusion. However, emerging evidence highlights its critical function in the formation and regulation of membrane contact sites (MCSs) between mitochondria and other organelles. We have been focusing on Mfn2-mediated interactions at three major types of MCSs: mitochondria–endoplasmic reticulum (ER), mitochondria–lipid droplets (LDs), and mitochondria–peroxisomes. At mitochondria–ER contact sites, Mfn2 contributes to calcium signaling, lipid exchange, and mitochondrial dynamics by tethering the two organelles. In mitochondria–LD interactions, Mfn2 facilitates lipid trafficking and energy metabolism, playing a crucial role in metabolic adaptation. At mitochondria–peroxisome interfaces, Mfn2 is implicated in coordinating fatty acid oxidation and reactive oxygen species (ROS) homeostasis. Together, these interactions underscore Mfn2’s multifaceted role in maintaining cellular homeostasis through inter-organelle communication. Understanding these mechanisms may provide new insights into the pathogenesis of metabolic diseases.

Endoplasmic Reticulum–Mitochondria Calcium Transfer: A Key Regulator in Cancer Cell Survival and Progression. [Julio Cesar Cardenas \(julio.cardenas@umayor.cl\)](mailto:julio.cardenas@umayor.cl).

Center for Integrative Biology, Faculty of Science, Universidad Mayor

The transfer of calcium ions (Ca^{2+}) between the endoplasmic reticulum (ER) and mitochondria is essential for regulating cellular homeostasis, metabolism, and survival. This finely tuned process occurs at specialized contact sites known as mitochondria-associated membranes (MAMs), where the inositol 1,4,5-trisphosphate receptor (IP3R) on the ER releases Ca^{2+} that is subsequently taken up by the mitochondrial calcium uniporter (MCU). In healthy cells, this Ca^{2+} flux supports mitochondrial bioenergetics by activating calcium-sensitive dehydrogenases of the tricarboxylic acid (TCA) cycle, thereby promoting ATP production and sustaining cellular function. In cancer cells, ER–mitochondria Ca^{2+} transfer sustains the elevated metabolic demands of tumor proliferation. Disruption of this axis, either pharmacologically or genetically by inhibiting IP3R or MCU, selectively induces cancer cell death, while normal cells adapt to the metabolic stress through compensatory mechanisms such as autophagy. Additionally, our work has uncovered a critical role for ER–mitochondria Ca^{2+} communication in both the establishment of therapy-induced senescence and the subsequent escape from senescence in cancer cells. In summary, ER–mitochondria Ca^{2+} transfer serves as a central regulator of cancer cell fate. Targeting this signaling pathway presents a promising therapeutic strategy for selectively eliminating tumor cells and preventing relapse by modulating both bioenergetic and senescence-related mechanisms.

LEAP-2 and Cardiac Dysfunction in MASLD: Exploring the Mitochondria–Lipid Droplet Axis in Metabolic Steatotic Liver Disease. [Valentina Parra](mailto:vparra@ciq.uchile.cl)^{1,2,3} (vparra@ciq.uchile.cl).

¹Laboratory for Cell Differentiation and Metabolism, Department of Biochemistry and Molecular Biology, Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile. ²Advanced Center of Chronic Diseases (ACCDiS), Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile. ³Systems Biology Center for the Study of Extremophile Communities from Mining Tailings (SYSTEMIX), O’Higgins University, Rancagua, Chile.

Cardiovascular diseases (CVDs) remain the leading cause of death worldwide, with mitochondrial dysfunction recognized as a central mechanism driving cardiomyocyte hypertrophy, impaired energetics and heart failure. Over the past years, our group has contributed to this field by uncovering how mitochondrial dynamics and function determine the balance between physiological and pathological remodeling in the heart, highlighting mitochondria as critical nodes not only for ATP production but also for signaling and organelle crosstalk that shape

cardiomyocyte fate. In hepatocytes, fatty acids distinctly remodel the mitochondria–lipid droplet (LD) axis, influencing whether mitochondria channel energy toward ATP synthesis or LD expansion, and this crosstalk has emerged as a determinant of lipid handling, oxidative stress, and organelle fitness, raising the possibility that its dysregulation contributes to systemic metabolic disease. Metabolic dysfunction-associated steatotic liver disease (MASLD), the most prevalent chronic liver disorder, provides a clinical context where these mechanisms become particularly relevant, as beyond hepatic lipid accumulation, MASLD alters the secretion of hepatokines that act systemically to influence cardiac metabolism and function. Among them, liver-expressed antimicrobial peptide 2 (LEAP-2), an endogenous antagonist of ghrelin, is markedly elevated in MASLD and may represent a novel mediator linking liver metabolism to cardiovascular dysfunction. Although evidence on its role in the heart is still limited, our recent findings suggest that LEAP-2 modulates lipid metabolism and mitochondrial dynamics in cardiomyocytes, promoting mitochondrial biogenesis but reducing membrane potential and counteracting the protective effects of ghrelin against lipid overload. Together, these observations suggest that disruption of the mitochondria-LD axis in MASLD, combined with elevated hepatokines such as LEAP-2, may provide a mechanistic framework to explain the increased cardiovascular risk associated with metabolic liver disease and highlight the mitochondria-LD axis as a promising target for future therapies.

Acknowledgment: This project is funded by ANID FONDECYT 1230195 (VP) and FONDAP 15130011 (VP).

Novel function and mechanism of action of proteins localized at the mitochondria-ER contact sites in regulating cellular senescence and age-related alterations. David Bernard (david.bernard@lyon.unicancer.fr). Equipe labellisée Ligue contre le Cancer; Senescence, Cancer and Aging team, Centre de Recherche en Cancérologie de Lyon, France.

Cellular senescence is activated in response to developmental signals or stresses during life. It is characterized by a stable arrest of proliferation and the acquisition of a senescence-associated secretory phenotype (SASP), composed of numerous factors, including pro-inflammatory molecules, proteases, and growth factors. Senescent cells can exert beneficial effects when timely regulated (e.g., embryonic development, wound healing), but become deleterious when they accumulate, particularly with aging and deleterious exposures (e.g., tobacco, certain diets, radiation). SASP affects the environment of senescent cells by inducing and modulating various phenotypes such as paracrine senescence, immune cell activity, and extracellular matrix deposition and organization, thus critically impacting various pathophysiological situations, including inflammation, cancer, and aging. We have identified the ER calcium release channel ITPR2 (inositol 1,4,5-triphosphate receptor type 2 or IP3R2) and the subsequent accumulation of mitochondrial calcium as an important mechanism promoting cellular senescence. Strikingly, aged *Itp2* KO mice exhibit lower cellular senescence, fewer signs of aging, and a longer lifespan than control mice. The ITPR2 channel also facilitates ER-mitochondria calcium transfer by promoting contacts, called MERC (Mitochondria-ER Contact), between these two organelles. We have recently screened approximately 100 MERC components to determine whether any of them can regulate cellular senescence. I will present published and unpublished results highlighting the importance of certain MERC components, including but not limited to ITPR channels, in regulating cellular senescence and certain senescence-associated alterations.

Simposium 6: “The importance of Protein Quality Control (PQC) *in vitro* and *in vivo*”

Organizers: Christian Wilson y Maximiliano Figueroa

Enhancing Research Data Reproducibility Through Protein Quality Control. André Matagne¹ (amatagne@uliege.be), Ario de Marco², Nick Berrow³, Mario Lebendiker⁴; Maria Garcia-Alai⁵, Stefan H. Knauer⁶, Blanca Lopez-Mendez⁷, Annabel Parret⁵, Kim Remans⁸, Stephan Uebel⁹, Bertrand Raynal¹⁰.

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The scientific community aims to enhance transparency and reliability in published research, with a specific focus on the quality of biological reagents, notably recombinant proteins. Concerns have been raised regarding irreproducible results, prompting discussions on common quality issues associated with recombinant proteins and their impact on downstream applications. In response, professionals in protein purification and characterization have collaboratively developed guidelines [1] for improved quality control. These guidelines include minimal information requirements for protein identity, production parameters, and long-term stability, as well as a minimal set of quality tests for purity, homogeneity, and identity. Additional recommendations cover DNA contamination, “spectral and thermal denaturation signatures”, homogeneity, “competent fraction”, storage conditions, batch-to-batch reproducibility, and other factors based on intended applications. Evaluation [2] of these guidelines over a one-year period suggests that their implementation can enhance experimental reliability and optimize protein quality. Investing in protein QC benefits all stakeholders in life sciences, including researchers, editors, and funding agencies, by improving data veracity and minimizing resource wastage. [1] de Marco, A., Berrow, N., Lebendiker, M., Garcia-Alai, M., Knauer, S.H., Lopez-Menez, B., Matagne, A., Parret, A., Remans, K., Uebel, S., Raynal, B. (2021) Quality control of protein reagents for the improvement of research data reproducibility, *Nat. Commun.* **12**, 2795. [2] Berrow, N., de Marco, A., Lebendiker, M., Garcia-Alai, M., Knauer, S.H., Lopez-Menez, B., Matagne, A., Parret, A., Remans, K., Uebel, S., Raynal, B. (2021) Quality control of purified proteins to improve data quality and reproducibility: results from a large-scale survey, *Eur. Biophys. J.* **50**, 453-460

Biophysical determination of free energy of protein folding at the single and multiple molecule level. Christian A.M. Wilson¹ (yitowilson@gmail.com), Camila Grazielle Corrêa^{1,2}, Are Mjaavatten³, Javiera Martínez-Bilbao^{1,4}, Karina New¹, Steven B. Smith⁵, Maximiliano F. Figueroa⁶.

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Free energy is a critical parameter in understanding the equilibrium in chemical reactions. It enables us to determine the equilibrium proportion between the different species in the reaction and to predict in which direction the reaction will proceed if a change is performed in the system. Historically, to calculate this value, bulk experiments were performed where a parameter was altered at a gradual rate to change the population until a new

equilibrium was established. In protein folding studies, it is common to vary the temperature or chaotropic agents in order to change the population and then to extrapolate to physiological conditions. Such experiments were time-consuming due to the necessity of ensuring equilibrium and reversibility. Techniques of single-molecule manipulation, such as optical/magnetic tweezers and atomic force microscopy, permit the direct measurement of the work performed by a protein undergoing unfolding/refolding at particular forces. Also, with the development of non-equilibrium free energy theorems (Jarzynski equality, Crooks fluctuation theorem, Bennett acceptance ratio, and overlapping method), it is possible to obtain free energy values in experiments far from equilibrium. This work compares different methodologies and their application in optical tweezers. Interestingly, in many proteins, discrepancies in free energy values obtained through different methods suggest additional complexities in the folding pathway, possibly involving intermediate states such as the molten globule. Finally, we use the artificial protein Top7 as an example of a metamorphic protein (that have different folded states). Our findings suggest that despite structural heterogeneity, Top7 returns to the folded state through a common route, hinting at conserved intermediate states.

Acknowledgment: ENLACE-FONDECYT FaCiQyF U. Chile

Direct observation of alpha-synuclein oligomerization on lipid membranes. Raman van Wee^{1,2,3} (raman.vanwee@sjc.ox.ac.uk), Dan Loewenthal^{1,2}, Gobert Heesink⁴, Mireille M. A. E. Claessens⁴, Andrew J. Baldwin^{1,2}, Richard Wade-Martins^{2,3,5}, Philipp Kukura^{1,2}.

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Introduction: The accumulation of α -synuclein oligomers is a hallmark of Parkinson's disease and the interaction with lipidic membranes is a key driver of their neurotoxicity. The low relative abundance of the oligomers combined with their non-amyloidogenic and transient nature hinder quantitative characterization of the oligomerization process.

Materials & Methods: Here, we use the label-free method mass photometry to capture the early-stage oligomerization of α -synuclein on supported lipid bilayers before this is detectable with Thioflavin-T staining.

Results: We show that monomers bind the membrane with micromolar affinity and subsequently get incorporated into growing oligomers in a first-order reaction that is polymorphous at the molecular level. By tracking the motility of oligomers we find that oligomers grow flat on top of the membrane and that targeting the protein-membrane or the protein-protein interaction with small molecules both effectively inhibit oligomerization. Lastly, we reveal the effect of familial Parkinson's mutations and the phosphorylation of serine129 on the earliest stages of aggregation.

Discussion: In this work we have characterized protein aggregation on lipid membranes at a length- and timescale inaccessible with conventional methods.

Acknowledgment: Wellcome Trust, Grant Number: 218514/Z/19/Z.

***N*-glycosylation and glycoprotein folding quality control in the fission yeast secretory pathway and insights in congenital disorders of glycosylation.** Cecilia D'Alessio (cdalessio@fbmc.fcen.uba.ar), Yommy Idrovo Hidalgo^{1,2}, Sofia Orioli¹, Cecilia D'Alessio^{1,2}. ¹Instituto de Biociencias, Biotecnología y Biología Traslacional, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. ²Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

Introduction: Protein *N*-glycosylation in the eukaryotic secretory pathway is essential for proper glycoprotein folding and function. This process begins in the endoplasmic reticulum (ER) with the transfer of the conserved glycan Glc₃Man₉GlcNAc₂ (G3M9) to asparagine residues of nascent proteins. The ER membrane protein glucosidase I (GI) trims G3M9, enabling further processing and access to ER quality control (ERQC), which

ensures that only correctly folded proteins advance along the pathway. Congenital Disorders of Glycosylation (CDG) are rare genetic diseases affecting protein glycosylation. GI deficiency causes MOGS-CDG, an autosomal recessive disorder with no effective treatment. Using *Schizosaccharomyces pombe* as a model, we previously showed that bypassing GI requirement by mutating the enzyme that adds the terminal glucose during synthesis of G3M9 failed to fully restore growth of GI-deficient (Δ GI) cells. This suggests that MOGS-CDG pathology may involve functions of GI beyond its enzymatic activity. Interestingly, although kinetoplastids never synthesize GI substrates, *Trypanosoma cruzi* retains a GI coding sequence.

Materials and Methods: To assess the role of GI membrane localization, we constructed a GI catalytic-domain-only variant (cGI) fused to GFP. Expression and localization of cGI in Δ GI yeast were confirmed by fluorescence microscopy and western blot. Enzymatic activity was tested *in vitro* with a fluorometric assay, and *in vivo* by metabolic radiolabeling.

Results: cGI displayed glucosidase activity *in vitro* and efficiently trimmed G3M9 *in vivo*, confirming activity and ER localization. However, Δ GI cells expressing cGI maintained the same sick phenotype as GI-null mutants.

Discussion: These findings suggest that GI's membrane localization or its transmembrane domain contribute to cellular fitness, providing insights into the molecular basis of MOGS-CDG.

Acknowledgment: ANPCyT PICT2020-A-3099

FREE COMMUNICATIONS ABSTRACTS

NEW MEMBERS SESSION

1. The mitochondria protease ClpP participates in VSMCs differentiation and modulates vascular diseases.

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Introduction: Vascular smooth muscle cells (VSMCs) exhibit remarkable phenotypic plasticity, a process central to vascular diseases such as atherosclerosis and aneurysms. We previously showed that deficiency of the mitochondrial protein Poldip2 enhances VSMC differentiation. Further analyses revealed that Poldip2 interacts with ClpX, the regulatory subunit of the ClpP protease, thereby limiting ClpP activity. Reduced Poldip2 expression results in ClpP activation. We therefore hypothesized that ClpP activation promotes VSMC differentiation and protects against atherosclerosis.

Methods: Human aortic SMCs (HASMCs) were used for in vitro studies. Gain- and loss-of-function approaches included siRNA-mediated silencing, adenoviral overexpression, and pharmacological activation of ClpP. VSMC phenotype was evaluated by measuring lineage marker expression. For in vivo studies, ApoE^{-/-} mice were fed a high-fat diet for 5 weeks, and atherosclerotic plaque burden was quantified by en face analysis.

Results: ClpP loss of function significantly impaired VSMC differentiation, whereas both genetic and pharmacological activation of ClpP enhanced differentiation. Mechanistic studies indicated that ClpP activation alters the cellular NAD⁺/NADH ratio and stimulates Sirtuin 1. Pharmacological activation replicated this phenotype in vitro and in vivo, leading to a marked reduction in plaque formation in ApoE^{-/-} mice.

Conclusion: These results establish mitochondrial proteostasis as a critical regulator of VSMC phenotype. They further identify the ClpP protease as a novel, actionable target for therapeutic modulation of VSMC plasticity and the treatment of vascular disease.

Acknowledgment: NIH HL095070, FONDECYT 1251373, AHA VPHA1474117.

2. DNA and histone modifications prevent heterochromatin dysfunction and reactivation of transposable elements (TEs) through different epigenetic mechanisms. Hugo Sepúlveda^{1,2} (hugo.sepulveda@unab.cl) and Anjana Rao².

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Introduction: DNA cytosine methylation (5mC) and demethylation are controlled by DNA methyltransferases (DNMTs) and TET (Ten-Eleven Translocation) methylcytosine dioxygenases, respectively. TETs (TET1/2/3) oxidize 5mC into 5-hydroxymethylcytosine (5hmC) and beyond, that are intermediate forms of the “passive/replication-dependent” and the “active” DNA demethylation pathways. The O-GlcNAc transferase OGT is an essential enzyme for proliferation and cell survival that robustly interact with all TET-enzymes and that modifies intracellular proteins with N-acetylglucosamine (O-GlcNAc). Here, we explored the connection between OGT, DNA modifications and TE-expression.

Methodology: We used *Ogt*^{fl}, *Cre-ERT2* mouse embryonic stem cells (mESC) to inducibly delete *Ogt*. The long-reads based method ONT, WGBS and the new method 6base-seq were used to measure DNA modifications (5mC/5hmC) genome-wide. RNA-seq was used for transcriptomic profiling of genes and TEs. co-immunoprecipitation coupled by Mass-Spectrometry (CoIP-MS) was used to determine the OGT-interactome.

Results: OGT-depletion results in a widespread increase in the TET product 5hmC in both euchromatin and heterochromatin, with concomitant reduction of 5mC. OGT-inhibition also displayed increased 5hmC, and attenuating the TET1-OGT interaction in mESC resulted in a genome-wide decrease of 5mC, indicating that OGT restrains TET activity and limits untoward DNA demethylation, requiring the TET-OGT interaction and OGT catalytic activity. DNA hypomethylation in OGT-deficient cells results in de-repression of heterochromatic TEs.

Discussion: We demonstrate that OGT protects the genome against TET-mediated DNA demethylation and loss of heterochromatin integrity, preventing the aberrant increase in TE-expression noted in cancer, autoimmune-inflammatory diseases, cellular senescence and ageing.

Acknowledgements: The PEW Charitable Trusts and California Institute for Regenerative Medicine (CIRM), US.

3. *ARIDIA* as a potential key regulator of tumor progression and treatment response in gallbladder cancer.

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Introduction: Gallbladder cancer (GBC) presents a high incidence and mortality rate in Chile. Due to late-stage diagnosis, fewer than 20% of patients are eligible for curative surgery, while the majority receive palliative chemotherapy, with a five-year survival rate below 5%. Among the mechanisms linked to tumor aggressiveness, the epithelial–mesenchymal transition (EMT) plays a central role, conferring invasive, migratory, and drug-resistant properties to tumors. Genomic studies have revealed recurrent alterations in *ARIDIA*, a subunit of the SWI/SNF chromatin remodeling complex. The aim of this work is to investigate the contribution of *ARIDIA* loss to the molecular mechanisms underlying tumor aggressiveness in GBC, with emphasis on EMT-related phenotypes.

Material and Methods: RNA-seq data from FFPE GBC samples were analyzed and stratified by *ARIDIA* expression (High vs Low). An *ARIDIA* knock-out cell line (GB-d1-KO) was generated using CRISPR/Cas9. Drug sensitivity to 5-FU and gemcitabine was assessed by MTS assays. Invasiveness was evaluated through Transwell-Matrigel assays. EMT-related gene expression (*MMP9*, *Vimentin*, *ZEB1*) was quantified by RT-qPCR.

Results: Low *ARIDIA* expression in patient samples was associated with enrichment of EMT and invasion pathways. In vitro, *ARIDIA* loss induced increased resistance to 5-FU (IC₅₀ WT vs. KO: 1.54 nM vs. 21.32 nM) and gemcitabine (IC₅₀ WT vs. KO: 0.43 μM vs. 1.47 μM), accompanied by enhanced migratory capacity and elevated expression of EMT markers.

Discussion: These results suggest that *ARIDIA* loss in GBC may promote invasiveness and chemoresistance, supporting its potential role as a prognostic and therapeutic biomarker in this malignancy.

Acknowledgement: Fondecyt Postdoctoral 3250470, Fondecyt Postdoctoral 3250447. Fondecyt 1221162, European Union's Horizon 2020 research and innovation program (grant 825741), FONDAP 152220002 (CECAN).

ORAL SESSION 1

OS1_1. Phylogenetic, Structural, and Functional Insights into the NSs Protein of the *Orthohantavirus andesense* (ANDV). Hade Ramos Acevedo^{1,4} (haramos@uc.cl), Eugenia Fuentes⁴, Andreas Schüller^{1,2}, Jorge Vera-Otarola³, Marcela Ferrés⁴ and Jenniffer Angulo^{1,4}.

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Introduction: The Andes virus (ANDV), a rodent-borne Hantavirus, causes hantavirus cardiopulmonary syndrome (HCPS) in Chile and Argentina. Its genome comprises three RNA segments: large (L), medium (M), and small (S). The S segment encodes the Nucleocapsid (N) and the Non-structural S (NSs) proteins. While N is crucial

in several stages of viral replication, NSs act as an antagonist of the type-I interferon (IFN-I) pathway, through interaction with MAVS, contributing to pathogenesis. This study examined the genetic diversity of the S segment and the functional impact of non-synonymous single nucleotide variations (SNVs) within the NSs protein.

Materials and Methods: Complete S segments from 58 ANDV-genomes collected from human clinical samples were sequenced with Illumina. Phylogenetic analyses were performed with QTREE. Structural predictions and stability analysis were conducted with Robetta, QUARK, SWISS-MODEL and FoldX. Artificial and natural occurring mutations were introduced by site-directed mutagenesis into the ANDV-HA-NSs plasmid, confirmed by Sanger sequencing, and functionally evaluated with an IFN- β promoter reporter assay in HEK293T cells.

Results: Phylogenetic analyses revealed two distinctive clades within Chilean isolates. Twenty-nine non-synonymous SNVs were identified, 62% within the NSs. Structural modeling and stability predictions highlighted a positively charged surface patch. Functional assays demonstrated that the artificial mutant, R4L-R5L, lost the ability to inhibit IFN- β activation, while the W28A and W42E did not. Among natural variants, the S32L mutation (detected only in a severe case) exhibited stronger IFN- β inhibition than the wild type, while the I20V and N34S did not.

Discussion: Phylogenetic and functional analyses indicate that the NSs is a hotspot for variability in ANDV. Specific mutations, such as S32L, modulate IFN antagonism, while R4L-R5L highlights a critical region for MAVS interaction. Identifying such critical regions may guide the development of therapeutic targets, supporting the protein's key role in host-virus interactions and its influence on disease outcomes.

Acknowledgment: FONDECYT N°1230718, N°1211825, N°1161167 and NIH ID14I10084.

OS1_2. Effect of Cold Shock Protein A on RNA Stability, Kinetics, and Misfolding Probability during Temperature Downshift. Rodrigo Rivera S.¹(rodrigo.rivera.s@ug.uchile.cl), Cristian Valdebenito¹, Cayetana Zamorano¹, Ernesto Roman², Gonzalo Cosa³, and Mauricio Baez¹

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Introduction: RNA folding has a rugged energy landscape that facilitates the formation of misfolded structures. A decrease in temperature exacerbates this problem by increasing the probability of kinetically trapped misfolded states. To overcome this situation, mesophilic microorganisms such as *Escherichia coli* overexpress Cold Shock Protein A (CspA), an RNA chaperone essential for bacterial growth at low temperatures. Here, we employed single-molecule and computational approaches to investigate how CspA modifies RNA folding and misfolding pathways under cold conditions.

Methods: We probed TAR RNA hairpin folding using optical tweezers between 10 and 30 °C. Optical tweezers are uniquely suited to reveal metastable states: in cyclic force ramps, misfolded conformations can persist during unloading and are revealed in subsequent loading phases by unfolding trajectories distinct from the native fold. To complement these assays, smFRET experiments were performed on a less-stable RNA hairpin (OldP) using TIRF microscopy to assess CspA effects in the absence of force. Additionally, a simplified statistical-thermodynamic model of RNA folding, based on nearest-neighbor base-pairing parameters, was used to evaluate the effect of cooling on the energy landscape.

Results and discussion: Cooling increased the frequency of misfolded states, whose unfolding patterns matched alternative conformations predicted as local minima by the thermodynamic model. Interestingly, CspA reduced RNA flexibility at low temperature and slowed folding kinetics, thereby decreasing the probability of misfolding in force spectroscopy experiments. In smFRET assays, CspA promoted the unfolded state of OldP in a concentration-dependent manner. The interplay between altered folding kinetics, binding to the unfolded state, and reduced flexibility induced by the chaperone suggests a novel mechanism of action, in which modulation of RNA plasticity contributes to the control of misfolding pathways during cold adaptation.

Acknowledgments: FONDECYT 1231276, Emerging Leaders in the Americas Program (ELAP), and Global Affairs Canada.

OS1_3. Improved microsome isolation from *Saccharomyces cerevisiae* enables peptidisc-based, detergent-free reconstitution of the Sec61 translocon. Alfaro-Valdés Hilda M.^{1,2} (hilda.m.alfaro.v@gmail.com), Andrea Aviles², Valentina Squicciarini², Bernardo Lopez², Aaron J. Paredes², Cecilia D'Alessio³, Christian A. M. Wilson². ¹Faculty of science, University of Valparaíso, Valparaíso, Chile. ²Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile. ³Institute of Biosciences, Biotechnology and Translational Biology (iB3). Department of Physiology, Molecular and Cellular Biology, Faculty of Exact and Natural Sciences, University of Buenos Aires, Buenos Aires, Argentina.

Introduction: We developed an optimized, high-yield method for isolating endoplasmic reticulum (ER)-derived microsomes from *Saccharomyces cerevisiae*. The protocol improves upon conventional preparations by combining mechanical disruption with glass beads and a simplified sucrose gradient, reducing both processing time and required culture volume while maintaining protein integrity.

Materials and Methods: For reconstitution, microsomes were solubilized using peptide technology, thereby avoiding exposure to detergents. The improved microsome isolation protocol yielded material well suited for detergent-free reconstitution of the Sec61 translocon using peptide technology.

Results: Mass photometry confirmed that the complex retained its native subunit composition and assembly. Additionally, Sec61 stability was observed in the presence of ATP γ S, supporting the functional integrity of the reconstituted complex.

Discussion: This optimized and reproducible protocol enables efficient detergent-free purification of membrane protein complexes, preserving their native state for structural and functional analyses. By combining enhanced microsome preparation with peptide reconstitution and affinity purification, we address common challenges in isolating and stabilizing fragile membrane proteins such as Sec61. The method can be readily adapted to other membrane systems, broadening its applicability across structural biology, membrane biochemistry, and biophysics. Overall, this approach offers a valuable tool for investigating membrane protein complexes under conditions that closely mimic their native environment.

Acknowledgment: ENLACE-FONDECYT Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.

OS1_4. Molecular characterization of *Botrytis cinerea* “Virulence Impaired Necrosis 1” (BcVin1) gene: Host-Dependent infection outcomes mediated by a transcriptional regulator of effector proteins. Gabriel Pérez-Lara^{1,2,3} (gabolev@gmail.com), Nicolás Arias-Inostroza^{1,2,3}, Valentina Hadler Palma^{1,3}, Danae Ramírez-Espinoza^{1,3}, and Paulo Canessa^{1,3}.

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Introduction: *Botrytis cinerea* is a fungus with significant commercial and biological importance. It causes "gray mold" disease in plants, leading to annual global economic losses of up to \$100 billion. Its broad host range has sparked increased interest in uncovering new infection strategies. Recent research emphasizes the role of a subgroup of effector proteins, known as cell-death-inducing proteins (CDIPs), in the infection process. These proteins can independently cause lesions in plant cells but remain poorly understood. Because the regulation of effector-coding gene expression in *B. cinerea* is unknown, we conducted a bioinformatic prediction of new effector proteins. When integrated into transcriptional networks, this data enabled us to identify the transcription factor (TF) Bcin03g00220 as a potential regulator of these proteins.

Materials and Methods: A Δ Bcin03g00220 loss-of-function mutant was constructed and tested for its infection ability on various plant hosts. RNA-seq, DAP-seq, and secretome analyses were performed to understand the role of Bcin03g00220 in effector regulation.

Results: Δ Bcin03g00220 produces reduced lesions on dicotyledonous leaves of beans, tobacco, tomato, and *Arabidopsis thaliana*, but not on monocotyledonous leaves. This TF was named Δ bcvin1 (Virulence Impaired Necrosis 1) because of its reduced ability to cause lesions. Transcriptomic analysis during plant infection showed a wide range of deregulated effectors in Δ bcvin1 during early and mid-to-late infection stages. However, DAP-seq

analysis indicated that BcVin1 is the primary regulator of effector expression in this late group of genes. Fungal proteomic analysis revealed massive differences in the protein profile, characterized by a decreased capacity to cause lesions in leaves in Δ b ν in1 compared to the wild-type strain.

Discussion: Collectively, these experiments demonstrate how BcVin1 regulates the expression of effector proteins during plant infection, underscoring the importance of these virulence factors and their precise temporal transcriptional coordination. The identification of early TFs regulating these molecules needs to be explored.

Acknowledgment: This research was funded by ANID-Ph.D. national scholarships 2021- 21210760 to G.P.-L. and by the ANID-FONDECYT grant numbers 1240742 to P.C.

OS1_5. Role of cyclooxygenases 1 and 2 in herpes simplex type 1-infected dendritic cells. [Areli J. Navarro¹ \(ahnavarro@uc.cl\)](mailto:ahnavarro@uc.cl), Mónica A. Farías¹, Felipe Cancino¹, Eduardo Tognarelli¹, Susan M. Bueno¹, Alexis M. Kallergis^{1,2}, Pablo A. González¹.

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Introduction: Herpes simplex virus type 1 (HSV-1) is a widespread pathogen that establishes lifelong infections in humans. Importantly, dendritic cells (DCs), key antigen-presenting cells that orchestrate antiviral immune responses, are susceptible to HSV-1 infection, which impairs their function and ultimately induces their death. Cyclooxygenases (COXs) are host enzymes that convert arachidonic acid into prostaglandins, including PGE₂, PGD₂, and thromboxane A₂, which strongly regulate inflammatory and immune processes. Previous studies suggest that COX-2-derived metabolites can suppress DC activity. The aim of this work was to investigate the role of COXs in HSV-1-mediated modulation of DC function.

Materials and Methods: We assessed COX expression in HSV-1-infected DCs using RT-qPCR and Western Blot. Pharmacological inhibition of COXs was used to evaluate their impact on DC viability, cytokine production, maturation, and T cell activation. Viral yield was also measured under COX inhibition.

Results: HSV-1 infection significantly modulates COX expression in DCs. Inhibition of specific COXs restored DC viability, modulated their cytokine profile and maturation state, and enhanced T cell activation. However, COX blockade did not affect HSV-1 virion production by DCs.

Discussion: These findings suggest that HSV-1 modulates COX expression in DCs, contributing to their dysfunction and death. Targeting COXs may represent a strategy to restore DC function during HSV-1 infection without compromising viral control.

Acknowledgment: Authors are supported by Fondecyt Regular 1240971 and the Millennium Institute on Immunology and Immunotherapy #ICN2021_045.

OS1_6. Variations in flanking or less conserved positions of the consensus binding sites for Reb1 and Abf1 transcription factors lead to major changes in their ability to modulate nucleosome sliding activity. [Fernanda Raiqueo \(fraiqueo2016@udec.cl\)](mailto:fraiqueo2016@udec.cl), Roberto Amigo and José. L. Gutiérrez.

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Introduction: Nucleosomes compact nuclear DNA and regulate its accessibility to DNA binding proteins. Among the factors that determine nucleosome positioning are chromatin remodeling complexes (CRCs) and transcription factors (TFs). Reb1 and Abf1 are yeast TFs which belong to a group of proteins called general regulatory factors (GRFs). They bind to specific DNA sequences and have been associated with hindering of nucleosome sliding mediated by CRCs such as ISW1a. In this context, it has been recently suggested that flanking nucleotides of binding motifs might influence protein binding affinity, binding kinetics and, in turn, TFs' functions. In this regard, we aimed to determine whether this type of sequence variations of Reb1 and Abf1 binding sites affect their ability to modulate ISW1a's sliding activity.

Materials and Methods: TFs were purified fused to His-tag from bacteria. ISW1a was purified by tandem affinity purification from Ioc3-TAP yeast strain. In vitro assays were performed using DNA probes reconstituted as nucleosomes, each displaying a different TF binding site variant. Affinity and dissociation rate constants (apparent K_d and K_{off} respectively) were determined for each variant, followed by nucleosome remodeling assays to test the ability of Reb1 and Abf1 to hinder ISW1a's sliding activity. Bioinformatics analyses were performed to analyze Reb1 and Abf1 binding sites found at gene promoters separately from gene bodies.

Results: Among the binding site variants tested for Reb1 and Abf1, those displaying the longest dwell time enabled them to hinder ISW1a's nucleosome sliding activity. The flanking nucleotides on these variants matched those enriched at gene promoter binding sites and differed from the ones present at gene bodies.

Discussion: Our results support a molecular function that requires high affinity and long dwell time binding sites, highlighting the relevance of determining the molecular functions that require and those that do not require these binding properties.

Acknowledgment: Universidad de Concepción VRID-Investigación 2023000737INV.

OS1_7. From Mapuche Medicine to Modern Science: Culén (*Otholobium glandulosum*) Antidiabetic Potential via In Vitro Enzyme Inhibition and Computational Screening. Muhammad Javid Iqbal^{1,2,3} (m.iqbal01@ufromail.cl), Cristian Paz² and Luis A Salazar³.

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Introduction: Chile confronts an escalating diabetes epidemic, with 1.9 million people currently affected and projections indicating a surge to 2.4 million cases by 2050. Traditional Mapuche medicinal plants, refined through centuries of indigenous wisdom, present untapped potential for addressing this healthcare crisis. This study bridges ancient knowledge with modern science by investigating the antidiabetic potential of four Mapuche medicinal plants through their inhibitory effects on key carbohydrate-metabolizing enzymes.

Materials and Methods: Four Mapuche plants-*Cryptocarya alba* (Peumo), *Otholobium glandulosum* (Culén), *Tristerix corymbosus* (Quintral de Maqui), and *Caldcluvia paniculata* (Triaca)-were evaluated for α -amylase and α -glucosidase inhibitory activities using in vitro enzymatic assays. The most active plant underwent gas chromatography-mass spectrometry (GC-MS) analysis for compound identification. Molecular docking studies were performed against α -amylase (PDB: 1B2Y) and α -glucosidase (PDB: 2ZEO) crystal structures.

Results: Culén demonstrated superior efficacy against both targets (IC_{50} : 82.7 μ g/mL for α -amylase; 27.4 μ g/mL for α -glucosidase), significantly outperforming acarbose (134.5 and 248.4 μ g/mL, respectively). GC-MS analysis identified 18 bioactive compounds, including bakuchiol derivatives, meroterpenoids, and furocoumarins. Molecular docking revealed 3-hydroxybakuchiol as the lead compound with exceptional binding affinities (-8.0 and -8.8 kcal/mol) through multiple molecular interactions.

Discussion: These findings validate centuries-old Mapuche therapeutic practices and position 3-hydroxybakuchiol as a promising natural antidiabetic agent, offering hope for culturally relevant, accessible diabetes management strategies for Chile's growing diabetic population.

Acknowledgment: ANID Scholarship (Folio 21252515).

OS1_8. Effect of 14-3-3 and DBC-1 interaction with ECE-1c in its protein stability in colorectal cancer cells. María de los Ángeles Toro¹ (maria.toro.b@ug.uchile.cl), Mariela González-Avendaño², Karla Villalobos-Nova¹, Paula Romero-Vicencio¹, Javiera Vargas¹, Ariela Vergara-Jaque², Julio C. Tapia¹.

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Introduction: The Endothelin-Converting Enzyme 1 isoform c (ECE-1c) has a role in cancer progression in different cancers, such as colorectal cancer. It is composed of three domains: an extracellular C-terminal catalytic domain, a transmembrane domain, and a short intracellular N-terminal domain. Phosphorylation of the N-terminus by protein kinase CK2 is related to an enhanced ECE-1c stability, as well as an invasive phenotype in colorectal cancer cells. Furthermore, expression of super-stable ECE-1c, with Lys-6 mutated to Arg, promotes stemness in glioblastoma, lung, and colorectal cancer cells. However, how ECE-1c phosphorylation translates in an enhanced stability remains unknown. One possibility is that a protein binds to the N-terminus of ECE-1c, blocking ubiquitination of Lys6 and proteosomal degradation. Indeed, 14-3-3 proteins bind to phospho-Ser/Thr residues of its targets, modulating its stability. Thus, we studied whether 14-3-3 interacts with ECE-1c and modulates its stability.

Materials and Methods: *In silico* analysis was performed to evaluate molecular docking of 14-3-3 proteins with phospho-ECE-1c. IP-MS/MS and pull down were then used to analyze predicted protein-protein interactions. siRNA specific for 14-3-3 and DBC-1 plasmid were used to evaluate ECE-1c protein levels by western blot.

Results: Molecular docking suggested that 14-3-3 g and e bind to phospho-Thr-9 of ECE-1c. Nevertheless, an IP-MS/MS analysis showed that both proteins do not differ in their interaction with wild-type or stable ECE-1c mutants. Moreover, siRNA specific for 14-3-3 isoforms did not affect the ECE-1c protein levels. Interestingly, DBC-1 (CCAR2) protein does interact with stable ECE-1c, which also modulated stability of ECE-1c^{WT} in colorectal cancer cells.

Discussion: DBC-1 by interacting with and enhancing ECE-1c stability, may be a key cofactor in promoting a malignant phenotype of colorectal and other cancer cells

Acknowledgement: ANID/FONDECYT #1220353

ORAL SESSION2

OS2_1. A Key Mitochondrial Player in Cellular Stress Resistance and Longevity. Andrea Matamoros¹, Juan Pablo Soffia¹, Marcelo Muñoz¹, Michael Maturana¹, Ma Andreina Rangel-Ramirez², Alvaro Gonzalez-Ibanez^{1†}, Gabriela Gomez-Lillo¹, Cesar Astorga^{1††}, Lina M. Ruiz³, Ramón Jorquera^{1¶}, Alejandra San Martín¹, Alvaro A. Elorza¹ (alvaro.elorza@unab.cl).

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Introduction: FAM162A is a mitochondrial protein evolutionarily conserved across taxa and ubiquitously expressed in various tissues. It is known for its role in hypoxia-induced apoptosis. However, paradoxically, FAM162A is overexpressed in cancer, where its pro-apoptotic function seems overridden, suggesting an alternative role associated with mitochondrial function and cell survival. Additionally, its precise localization and topology remain controversial. We aimed to assess the role of FAM162A in mitochondrial structure, dynamics, and bioenergetics and its impact on cell viability, while establishing its precise localization, orientation, and topology. Additionally, to generate a transgenic *Drosophila* model overexpressing human FAM162A to evaluate its effects on organismal survival under normal and stress conditions.

Methods: Localization, orientation, and topology were determined by protease protection assays in COS7 cells. Loss- and gain-of-function experiments were performed to assess cell viability, mitochondrial function and turnover by confocal microscopy, immunoblots and Seahorse technology. A transgenic *Drosophila* model overexpressing human FAM162A was generated to evaluate organismal survival under normal and stress conditions.

Results: FAM162A supports mitochondrial ultrastructure and bioenergetics, thereby influencing cell viability and stress resistance. It resides predominantly in the inner mitochondrial membrane, particularly within the cristae playing a role in cristae remodeling through the interaction and modulation of the fusion protein OPA1. Transgenic *Drosophila* overexpressing human FAM162A exhibited increased lifespan and locomotor activity under both normal and heat stress conditions.

Conclusion: FAM162A emerges as a crucial player in maintaining mitochondrial integrity and bioenergetics. Its functional role, through regulation of OPA1, impacts mitochondrial health, stress resistance, cellular viability, and organismal longevity.

Acknowledgment: FONDECYT 1251799 and 1180983; FONDEQUIP EQM220115; UNAB DI-03-24/IA; UNAB DI-03-22/NUC; UNAB DI-13-22/INI and DI-07-22/INI; NINDS-5R01NS108778-04; ANID Ph.D. Scholarship 2021-21212271 (AM); UNAB Ph.D. Scholarships (GGL and JPS).

OS2_2. Antisense oligonucleotides directed to the SARS-CoV-2 genome reduce viral load *in vitro* and *in vivo*. Christopher Fitzpatrick^{1,2} (csfitzpatrick@gmail.com), Sophie Dhorne-Pollet² Nicolas Meunier², Sophie Lepoder³, Constanza Gabilán¹, Maximiliano López-Muñoz¹, Juan Pablo Barraza¹, Bernard Delmas², Eric Barrey² and Verónica A. Burzio¹.

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Introduction. SARS-CoV-2 is an RNA virus responsible for the COVID-19 pandemic. The limited efficacy of current antivirals, the long-term sequelae in patients, and their association with cancer highlight the need for new and more effective therapeutic strategies. We tested the ability of antisense oligonucleotides (ASOs) to target different viral sequences in cell and animal infection models.

Methods. Phosphorothioate ASOs targeting SARS-CoV-2 RNA sequences were designed based on viral genome accessibility and tested in infected HEK-293t/ACE2 cells using reporter assays and qPCR. The best candidate ASO was validated in SARS-CoV-2-infected Syrian Golden hamsters, a natural host for the virus. Other ASO chemical modifications and combinations of them were tested.

Results: The ASO directed to the nucleocapsid region of the SARS-CoV-2 subgenomic RNAs (ASO-N1) exhibited the best antiviral effect *in vitro*. SARS-CoV-2-infected animals treated with ASO-N1 showed a reduction in viral load and displayed better clinical outcomes and inflammatory cytokine profiles than control animals. A 1:1 combination of Phosphorothioate and Morpholino-modified ASO-N1 improved antiviral efficacy.

Discussion. The SARS-CoV-2 nucleocapsid RNA can be used as an effective target for ASO therapy, since this sequence is highly conserved among known SARS-CoV-2 variants. ASO-N1, directed to the nucleocapsid region, proved to be the most effective *in vitro* and decreased viral titers *in vivo*, corroborating this region as a strong target. A combination of ASOs with different mechanisms of action suggests a synergic antiviral effect. Further research derived from this work involves host microRNAs as biomarkers and potential targets for antiviral strategies. ASO therapy is a potentially powerful approach to treating SARS-CoV-2 infection and other viral diseases, which could prevent disease progression and long-term sequelae at the early stages of infection.

Acknowledgements: ANID, Chile, Fondecyt-1230760; European Union Horizon 2020, Marie Skłodowska-Curie grant 945298-ParisRegionFP; French National Research Agency RA-COVID-19 Grant.

OS2_3. A *KCNK1* Variant Linked to Rett Syndrome Disrupts ER to Golgi Trafficking of Kv3.1 Channel. Diego Maureira¹ (diego.maureira.f@ug.uchile.cl), Carla Rubilar², Joaquín López¹, Paola Santander², Hans Moldenhauer^{1,3}, Ian Silva^{1,4}, Pablo Cruz¹, Denise Riquelme⁵, Javiera Baeza^{1,6}, Wendy González⁶, Evrim Servili¹, Mónica Troncoso^{2,#}, Elías Leiva-Salcedo^{5,#} and Oscar Cerda^{1,#}.

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Introduction: Intrinsic neuronal excitability, defined by the balance between input and output signals, is crucial to neural function, and its disruption underlies various neurological diseases. Kv3.1 channels, encoded by *KCNK1*, are essential for high-frequency action potential firing. Variants in these channels are associated with several

subtypes of epilepsy. We report a patient with developmental regression and epilepsy, meeting Rett syndrome criteria, who carries a *KCNK1* variant encoding the S474C substitution in Kv3.1 (Kv3.1^{S474C}).

Materials and Methods: We performed patch-clamp, surface biotinylation and trafficking synchronization assays in cellular models to evaluate the trafficking and presence of the mutant channel in the plasma membrane. Moreover, we used voltage-clamp and immunofluorescence assays in primary cortical neuron cultures to assess the activity and localization of the Kv3.1^{S474C} channel in a physiological context. Finally, we performed molecular dynamics simulations to evaluate the stability of the Kv3.1^{S474C} channel tetramer.

Results: Electrophysiological and biochemical assays reveal that Kv3.1^{S474C} reduces channel presence in the plasma membrane and is retained in the endoplasmic reticulum (ER). In murine primary cortical neuron cultures expressing Kv3.1^{S474C}, we observed a reduction in neuronal firing frequency and exclusion of the channel from the axon initial segment (AIS). Additionally, molecular dynamics simulation indicates a reduction in the free energy when Kv3.1^{S474C} channel was evaluated.

Discussion: In summary, this study identifies a novel link between a *KCNK1* variant and Rett syndrome, highlighting the importance of S474 residue in Kv3.1 channel trafficking and function in neurons.

Acknowledgment: This research was funded by FONDECYT Grant 1240633 (ANID), FIA 2024 (from ICBM-HCUC, Universidad de Chile) (to OC), the Millennium Nucleus of Ion Channel-Associated Diseases (to OC and WG), FONDECYT Grant 1220680 (ANID) (to ELS) and Proyecto Ayudante_DICYT, (#022543LS_ayudante), Vicerrectoría de Investigación, Innovación y Creación (to ELS), USACH. MiNICAD is a Millennium Nucleus supported by the Iniciativa Científica Milenio, ANID, Chile. FONDECYT Grant 11251815 (ANID) funded HM. FONDECYT Grant 1230446 funds WG. DM, JL, and JB are recipients of ANID Doctoral Fellowships (#21201941, #21230481 and #21210923, respectively). ANID Postdoctoral Fellowship 3240187 funds to ES.

OS2_4. Dissecting pattern-triggered immunity in sweet cherry: contrasting responses to bacterial and fungal MAMPs. Andree Álvarez^{1,3,8} (andree.alvarez@postdoc.uoh.cl), Mariela González^{1,3}, Uri Aceituno-Valenzuela^{1,2,3}, Daniela Muñoz^{1,3}, Paz Contreras-Lopez^{1,3}, Franco Figueroa¹, Manuel Pinto¹, Meirav Leibman-Markus⁴, Mauricio Latorre^{2,3,6,7}, Victor Aliagada⁹, Alexander Vergara⁹, Luis Villalobos^{10j}, Paula Pimentel¹⁰, Adi Avni⁵, Maya Bar⁴, Claudia Stange⁸, Lorena Pizarro^{1,2,3}

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Introduction: Pattern-triggered immunity (PTI) represents a conserved branch of the plant immunity; yet little is known about how signaling and MAMP-specific variability are orchestrated in woody crops. Sweet cherry (*Prunus avium* cv. Lapins), challenged by important pathogens such as *Pseudomonas syringae* pv *syringae* and *Botrytis cinerea*, provides a model to study MAMP-specific immunity for resistance-based management strategies.

Methods: We functionally characterized the cherry FLS2 ortholog (PaFLS2) through cloning, localization, and oxidative burst measurement, and compared its 3D structure to PaEIX2 using IsoformMapper. *P. avium* PTI response was studied using detached leaves challenged with bacterial flg22 or fungal xyn11/EIX. Induced resistance to *Pseudomonas syringae* pv *syringae* and *Botrytis cinerea*, and expression of 26 defense- and metabolism-related genes were quantified at 6 and 24 h. A *P. avium* regulatory network was constructed based on *Arabidopsis* data. In addition, targeted phytohormone metabolomics was performed to quantify SA, JA, ABA, GA, auxins (LC-MS/MS) and ET (GC).

Results: PaFLS2 localized to the plasma membrane and triggered oxidative bursts. Pathogen assays showed both flg22 and xyn11/EIX reduced *P. syringae* lesions, but only xyn11/EIX protected against *B. cinerea*.

Transcriptional responses to the MAMPs reflected MAMP specificity. Some genes exhibited opposite regulation between the MAMPs (*PaβLCY1*, *PaACO2*; *PaPAL1*). Network analysis identified WRKY, MYB, and AP2/EREBP transcription factors as central hubs linking PRRs, ABA metabolism (*PaAAO3*, *PaNCED3*), and stress genes (*PaGST*). Hormonal profiling revealed xyn11/EIX induced a broad defense signature (SA, JA/JA-Ile, ET, GAs, auxins), while flg22 selectively altered GA homeostasis.

Discussion/Conclusion: Bacterial (flg22) and fungal (xyn11/EIX) elicitors activate distinct PTI programs in cherry. Flg22 restricts bacterial infection alongside GA modulation, whereas xyn11/EIX drives multi-hormonal, transcriptional reconfiguration and broad protection. These elicitor-specific signatures offer potential for resistance-based crop protection in stone fruits.

Acknowledgment: ANILLO ACTO190001, Anillo ACT192073, PAI7190027, ID23110052, Centro UOH de Biología de Sistemas para la Sanidad Vegetal (BioSaV) - CI2302.

OS2_5. Microarchitecture of Dentin Analogs: Exploring Glycation in Oral Disease Models. Carmen Hidalgo¹ (carmen.hidalgo@uc.cl), Paula Ford¹, Carla Inostroza¹, and Sebastián Aguayo^{1,2}.

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Introduction: Oral conditions like caries and periodontal disease are prevalent conditions that worsen with age and contribute to significant oral health deterioration worldwide. The advancement of these diseases is driven by intricate interactions between the host and microbes. These interactions are intensified by age-related changes, such as glycation of the extracellular matrix and cellular aging, which contribute to tissue damage. Although there is increasing evidence connecting glycation and cellular aging to the pathogenesis of these oral diseases, their precise roles, particularly in aging tissues, are not well defined. Here, we aim to develop a bioengineered dentin analog model using direct 3D bioprinting to investigate the impact of dentin analog glycation on bacterial biofilm formation and cellular senescence.

Materials and Methods: A dentin analog model was fabricated using the PRIMO 2 photo-patterning system with varying UV dosages and PhotoCol®. The resultant dentin analog constructs were mineralized and glycated to simulate aging, and their architecture was characterized using optical and confocal microscopy. Pathogenic biofilms of *Streptococcus mutans* and *Candida albicans*, were grown on the glycated and non-glycated dentin analogs for 24 hours and examined by epifluorescence microscopy.

Results: The dentin analogs were successfully developed through 3D bioprinting, exhibiting structural features closely resembling human dentin with tubules of 2 μm of diameter arranged in both organized and disorganized patterns. The constructs exposed to a 1500 mJ/mm² UV dose exhibited a significantly greater height and supported the seeding of oral microorganisms. Microscopic images confirmed successful biofilm formation on glycated and non-glycated constructs, with the model replicating the aged microenvironment.

Discussion: The present study provides a scalable and precise model for oral disease research, with implications for understanding host-pathogen interactions and the effects of aging on oral tissues.

OS2_6. A Novel Catalytic Strategy for Phosphate Condensation: Insights from HMP-P Phosphorylation.

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Introduction: Phosphoryl transfer reactions mainly involve the transfer of a phosphoryl group from ATP to an acceptor hydroxyl group, which requires prior deprotonation of the hydroxyl, facilitated by solvent, the ATP substrate, or a catalytic base. The activated nucleophile then attacks the γ-phosphate of ATP via either an associative pathway with a pentavalent phosphate intermediate or a dissociative pathway involving a metaphosphate intermediate.

In bacterial vitamin B1 biosynthesis, 4-amino-2-hydroxymethylpyrimidine (HMP) undergoes two sequential phosphorylation steps to form HMP-PP through the intermediate HMP-P, both catalyzed by ThiD-HMPPK enzymes. This kinase belongs to the Ribokinase superfamily, where a conserved aspartate in the GXGD motif usually acts as a catalytic base. Interestingly, in ThiD-HMPPK, this aspartate is absent, and instead, a cysteine occupies the equivalent position. Moreover, HMP-P phosphorylation is unique within the superfamily, as it employs a methyl phosphate (R-CH₂-PO₄) as the acceptor group instead of a primary alcohol.

Materials and Methods: We crystallized an ancestral ThiD-HMPPK from Enterobacteriales (ancEn-ThiD) in complex with HMP or HMP-P and non-hydrolyzable ATP analogs, performed site-directed mutagenesis of active-site residues, and evaluated alternative mechanisms through QM/MM simulations with the Adaptive String Method.

Results and Discussion: HMP deprotonation occurs via solvent, while HMP-P deprotonation is assisted via substrate, with both reactions proceeding through dissociative pathways. HMP-P phosphorylation is facilitated by residues H179 and T211, which stabilize ATP, while H209 stabilizes the phosphate group of HMP-P. Site-directed mutagenesis further supports these findings: the C213A mutant was inactive with HMP and HMP-P, whereas C213D enhanced HMP phosphorylation by increasing k_{cat} 6-fold without significantly affecting HMP-P activity. In contrast, K111A demonstrated that lysine is essential for HMP-P phosphorylation (k_{cat} decreased 10-fold) but less critical for HMP (k_{cat} decreased 2-fold). These findings unveil a novel phosphoryl condensation mechanism, expanding catalytic diversity within the Ribokinase superfamily.

Acknowledgment: Beca Doctorado ANID folio 21221449 & Ayuda de viaje Departamento de Biología, Facultad de Ciencias, Universidad de Chile (N. Fuentes-Ugarte). Fondecyt 1221667 (IP: Víctor Castro-Fernandez) & Fondecyt 1230999 (IP: J Alzate-Morales).

OS2_7. Structural and evolutionary insights into the allosteric regulation and K⁺-independent activity in pyruvate kinases from methanogenic archaea. Sebastián M. Muñoz¹ (sebastian.munoz.m@ug.uchile.cl), Ignacio Aravena-Valenzuela¹, Antonia Alarcón-Saavedra¹, Nicolás Fuentes-Ugarte¹, Guy Schoehn², Víctor Castro-Fernández¹, and Victoria Guixé¹.

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Introduction: Pyruvate kinase (PK) catalyzes the final step of glycolysis, converting phosphoenolpyruvate (PEP) and ADP into pyruvate and ATP. Although PKs are homologous across all domains of life, their allosteric regulation shows remarkable diversity. In eukaryotes and many bacteria, fructose-1,6-bisphosphate (FBP) acts as an activator, whereas in other bacteria and in methanogenic archaea, AMP fulfills this role. Moreover, PKs exhibit an almost dichotomic dependence on potassium ions: in K⁺-dependent enzymes, a Glu residue is present at the active site, whereas in K⁺-independent enzymes this position is occupied by Lys. To date, no structural basis has been established for the evolution of the allosteric regulation in PKs from methanogenic archaea, nor has the structural basis for K⁺-independent activity been elucidated.

Materials and Methods: We employed ancestral sequence reconstruction to trace the evolutionary origin of AMP specificity at the allosteric site of PKs in methanogenic archaea. A phylogenetic tree comprising 411 PK sequences enabled the inference of a common ancestor of extant PKs regulated by AMP and FBP (AncCPK), and the last common ancestor of the order *Methanococcales* (AncMcPK). Both ancestral PKs were recombinantly expressed, purified, and structurally characterized by cryo-EM, together with extant PKs from *Methanococcus maripaludis* and *Methanocaldococcus jannaschii*.

Results and Discussion: In all cases, AMP binds at the allosteric site, indicating that this site is an ancestral trait. The 3.0 Å structure of AncCPK revealed a Glu residue at the active site coordinating an ion density at the K⁺ site. By contrast, the high-resolution 2.1 Å structure of AncMcPK showed a Lys residue coordinating a water molecule, functionally replacing K⁺ in the stabilization of PEP and thereby providing a structural basis for K⁺-independent activity. Moreover, the apo state of AncMcPK revealed significant conformational changes upon AMP and PEP binding, including lid-domain closure and loop rearrangement within the allosteric site.

Acknowledgment: Beca Doctorado Nacional folio N°21230418 & Ayuda de viaje Departamento de Biología, Facultad de Ciencias, Universidad de Chile (Sebastian M. Muñoz), FONDECYT 1231263 (Victoria Guixé).

OS2_8. DRP1 and Bcl-xL interaction in therapy-induced senescence progression in a model of colorectal cancer. Pablo Morgado-Cáceres^{1,2,3,4} (pablo.morgado@ug.uchile.cl), Donato Garrido¹, Osmán Díaz-Rivera¹, Andrea Puebla-Huerta^{1,4}, Ulises Ahumada-Castro^{1,4}, Eduardo Silva-Pavez¹, Hernán Huerta¹, Daniela Moreno¹, Valentina Castillo¹, César Casanova¹, Sergio Linsam Barth¹, Valentina Parra^{2,3,5}, César Cárdenas^{1,4}.

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Introduction: Colorectal cancer (CRC) is one of the leading causes of cancer-related mortality. Chemotherapy is designed to induce CRC cell death; however, a fraction of cells evades apoptosis and enter therapy-induced senescence (TIS), a state of stable cell cycle arrest with a distinctive secretome and other features. In several cancers, low DRP1, a key mitochondrial fission protein, has been associated with increased chemotherapy susceptibility, suggesting that reduced fission contributes to this effect, although consensus is lacking. Notably, DRP1 interacts with the anti-apoptotic protein Bcl-xL, highly expressed in senescence models. We hypothesized that DRP1 interacts with Bcl-xL during TIS, favoring senescence over cell death.

Materials and Methods: Human CRC cell lines HCT-116 and DLD-1, including DRP1 and MFF knockouts, were used. TIS was induced with doxorubicin (62.5 μ M, 48 h). Survival and senescence were evaluated by crystal violet staining, flow cytometry, SA- β -gal activity, and Western blot of senescence proteins. Pharmacological inhibition of DRP1 and Bcl-xL was also tested. DRP1/Bcl-xL spatial association was assessed by proximity ligation assay.

Results: Doxorubicin efficiently induced TIS in CRC cell lines. Genetic ablation of DRP1, but not its pharmacological inhibition, promoted cell death during this process, whereas MFF loss did not. TIS induction was accompanied by increased DRP1/Bcl-xL proximity. Notably, wild-type and MFFKO cells displayed minimal sensitivity to Bcl-xL inhibition under doxorubicin treatment, while DRP1KO cells exhibited markedly reduced survival, supporting a cooperative role of DRP1–Bcl-xL interaction in TIS establishment.

Discussion: DRP1 appears essential for CRC cell survival during TIS induction through mechanisms independent of mitochondrial fission. To our knowledge, this is the first report describing DRP1/Bcl-xL interplay in TIS, highlighting a potential novel axis in senescence progression.

Acknowledgment: ANID-FONDECYT 1230195 (VP) and 1240807 (CC); FONDAP 15130011 (VP) and 15150012 (CC); and ANID-Scholarship 21212019 (PMC).

POSTERS ABSTRACTS

P1. Mitochondrial OGG1 overexpression attenuates oxidative stress-induced senescence in HepG2 cells. Valentina Abarca¹ (valentina.abarca.v@ug.uchile.cl), Xavier Vielma¹, Lautaro Magaña¹, Emanuel Guajardo^{2,3,4}, Valentina Parra^{3,4,5}, Roberto Bravo-Sagua^{1,4,5}.

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Introduction: Aging increases the incidence of metabolic dysfunction-associated steatotic liver disease (MASLD), a process linked to oxidative stress-induced cellular senescence. As suggested in other models, this

condition is accompanied by inflammation, mitochondrial DNA (mtDNA) damage, and its release into the cytosol. The DNA repair enzyme OGG1 removes oxidized bases from mtDNA, and its mitochondrial overexpression has shown protective effects against inflammation; however, its role in human hepatocytes remains unknown. This study aimed to evaluate whether mitochondrial OGG1 overexpression protects against oxidative stress-induced cellular senescence in the HepG2 cell line.

Materials and Methods: Cellular senescence was induced in HepG2 cells by treatment with H₂O₂ (50 and 100 μM, 1 h) followed by 72 h recovery. The model was validated by assessing p16, p21, and p53 protein expression (Western blot), β-galactosidase activity, and pro-inflammatory cytokine mRNA expression (RT-PCR). Mitochondrial dysfunction was evaluated by membrane potential (immunofluorescence) and oxygen consumption (Clark electrode oxygraphy). mtDNA release into the cytosol was measured by cellular fractionation and real-time PCR. Finally, mitochondrial OGG1 overexpression was achieved by plasmid transfection, and its effect on senescence was determined through β-galactosidase activity.

Results: H₂O₂ treatment induced senescence in HepG2 cells, accompanied by mitochondrial dysfunction and mtDNA release into the cytosol. Overexpression of mitochondrial OGG1 reduced the proportion of β-galactosidase-positive cells, demonstrating a protective effect against oxidative stress-induced senescence.

Discussion: Mitochondrial OGG1 overexpression seemingly prevents the development of cellular senescence in HepG2 cells under oxidative stress; however, further experiments are required for confirmation.

Acknowledgements: This work was supported by Universidad de Chile (ENL09/24 and 2024P1DID, RB), ANID FONDECYT 1230195 (VP), and FONDAF 15130011 (VP, RB).

P2. Glycated microenvironments enhance *Candida albicans* – *Streptococcus mutans* synergy in dual-species biofilms. Alvarez, Simon^{1,2}(sealvarez1@uc.cl); Ortiz, Javiera¹; Berrios, Pablo²; Inostroza, Carla²; Ravasio, Andrea²; Aguayo, Sebastian^{2,3}.

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Introduction: Dental aging is associated with biochemical changes in dentin, including the accumulation of advanced glycation end-products (AGEs) that alter its mechanical and adhesive properties. These modifications may influence the development of cariogenic biofilms, particularly those formed by *Streptococcus mutans* and *Candida albicans*, which frequently co-occur in dental root caries. This study aimed to evaluate the impact of glycated collagen microenvironments affecting dual-species biofilm formation and structure.

Materials and Methods: To mimic aged dentin, microfabricated polydimethylsiloxane (PDMS) surfaces were coated with type I collagen, mineralized with hydroxyapatite, and treated with methylglyoxal (MGO 10mM) to induce collagen glycation. Dual-species biofilms were seeded on control and glycated substrates, and their development was assessed using confocal microscopy, atomic force microscopy (AFM), and RT-qPCR.

Results: Structural analysis revealed that glycated surfaces promoted denser, thicker biofilms with increased extracellular polymeric substance (EPS) accumulation, as confirmed by 3D reconstructions using SYTO9, CalcoFluor White, and Alexa 647-dextran staining. AFM measurements demonstrated greater stiffness in glycated substrates, which correlated with enhanced microbial adhesion. Preliminary RT-qPCR data showed altered gene expression in both species, including upregulation of ALS3 and Hwp1 in *C. albicans*, suggesting a shift towards a more pathogenic phenotype.

Discussion: These findings indicate that glycation-induced changes in the dentin microenvironment enhance the cooperative behavior of *S. mutans* and *C. albicans*, supporting a synergistic biofilm phenotype with potential implications for caries progression in elderly patients.

Acknowledgments: FONDECYT regular #1220804, #1210872, FONDEQUIP #EQM210101, Beca Doctorado Nacional ANID.

P3. Differential effect of water deficit and exogenous nitric oxide on the Lateral Root formation of three tomato cultivars (*Solanum lycopersicum*). Nicolás Arenas¹ (nicolas.arenas@pregrado.uoh.cl), Lorena Pizarro^{1,2}, Carlos Rubilar-Hernández¹.

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Introduction: Drought is a decrease in water availability caused by a reduction in soil moisture which significantly impacts crop growth. One adaptative response to this challenge is the modulation of Lateral Root formation (LRf). Depending on its intensity, LRf is modulated: it is inhibited in an air patch, nevertheless moderate water deficit (WD) stimulates it. In this context, nitric oxide (NO) emerges as a factor worth considering: it is a signaling molecule that increases its levels under WD and participates in LRf at optimal irrigation by interacting with auxin signaling - a key phytohormone involved in LRf. This study evaluated the effects of WD and exogenous NO in LRf of three tomato varieties.

Materials and Methods: M82, Moneymaker and Rosado tomato seedlings were grown in soil (peat:perlite:vermiculite 2:1:1) under three water regimes to configure optimal irrigation (85-95% saturation weight [SW]), mild (65-75% SW) and moderate WD (45-55% SW). Additionally, seeds were exposed to 0.1-0.5 mM Sodium nitroprusside (SNP), a NO donor. Seedlings were cultivated under optimal irrigation and moderate WD. One-month-old roots were clarified and LR events were counted using an optical microscope.

Results: Total LR events significantly increased under moderate WD in Moneymaker and Rosado varieties primarily due to LR primordia induction. Moreover, SNP treatment could induce LR primordia only in these two varieties.

Discussion: These results suggest that exogenous NO could promote LRf in tomato, resembling moderate WD effects. This highlights NO as a potential key player in LRf under WD. The differential response observed among tomato varieties indicates that a NO-dependent mechanism is functional only in certain varieties which could be a promising strategy for breeding focused on WD mitigation. Overall, these findings open new avenues for exploring molecular players involved in NO homeostasis that could influence LRf in crops under water stress.

Acknowledgment: FONDECYT_11251267; BioSav_CI2302.

P4. Design of interfering competitive peptides based on TRPM4-KCTD5 interaction. Javiera Baeza¹ (javierabaezajara@gmail.com), Diego Maureira², Ian Silva³, Wendy Gonzalez¹, Oscar Cerda².

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Introduction: The high expression of the Transient Receptor Potential Melastatin 4 channel (TRPM4) has been reported to be associated with pathologies that alter cytoskeletal rearrangements and cell migration, such as metastatic cancer in various cell lines. KCTD5 was identified as a positive regulator of TRPM4, causing increased Ca²⁺ sensitivity and involvement in cell migration through the regulation of TRPM4. TRPM4 and KCTD5 expression levels are elevated in breast cancers with poor prognosis. Therefore, TRPM4-KCTD5 interaction represents an attractive target for the development of peptides that modulate TRPM4 activity with therapeutic potential.

Materials and Methods: The peptides designed *in silico* were evaluated through *in vitro* assays in HEK293^{KCTD5} and MDA-MB231 cells, including intracellular sodium recordings, immunofluorescence, cell invasion, and Bimolecular Fluorescent Complementation (BiFC).

Results: A significant decrease in sodium influx was observed in HEK293 cells treated with the peptides TAT-TRPM4-HA and TAT-KCTD5-HA compared to the untreated control. In addition, a significant decrease in cell invasion was observed in MDA-MB-231 cells treated with the peptide TAT-TRPM4-HA compared to the control. In BiFC assays, a reduction in the fluorescence of TRPM4-KCTD5 complexes was observed when treated with the peptides TAT-TRPM4-HA or TAT-KCTD5-HA.

Discussion: Together, these experiments suggest that peptides designed *in silico* inhibit the TRPM4-KCTD5 interaction.

Acknowledgment: FONDECYT N° 1230446 and N° 1240633.

P5. Antihypertrophic Role of Estrogen/VCAM-1 in the Regulation of Cardiac Hypertrophy in Neonatal Rat Cardiomyocytes. Elsa Rocío Bascuñán^{1,2}(elsa.bascunan@ug.uchile.cl), Mayarling F Troncoso^{1,2}, Magda C. Díaz-Vesga^{1,2}, Danica Jimenez-Gallegos^{1,2}, Mario Chiong^{2,3}, Sergio Lavandero^{1,2}.

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Background: Cardiovascular diseases are the leading cause of mortality worldwide, with hypertension as a major risk factor. Postmenopausal women are particularly susceptible to left ventricular hypertrophy (LVH), a maladaptive response associated with diastolic dysfunction. Estradiol (E2) confers cardioprotection, although the underlying molecular mechanisms remain unclear. Our group recently identified vascular cell adhesion molecule-1 (VCAM-1) expression in cardiomyocytes, suggesting a potential protective role during cardiac stress.

Objective: To evaluate whether the estradiol/ER α axis prevents angiotensin II (Ang II)-induced cardiomyocyte hypertrophy by regulating VCAM-1 expression.

Methods: Primary neonatal rat ventricular myocytes (NRVMs) were exposed to Ang II (0.5–1 μ M for 24–48 h) to establish a hypertrophy model. Cells were pretreated with E2 (100 nM for 6 h) prior to Ang II stimulation. Hypertrophic markers (ANP, BNP, β -MHC) and VCAM-1 levels were assessed by qPCR and Western blot.

Results: Ang II at 1 μ M for 48 h significantly increased cardiomyocyte area and perimeter, confirming hypertrophy. E2 pretreatment reduced Ang II-induced ANP and BNP expression and significantly decreased β -MHC protein levels. VCAM-1 expression tended to increase under Ang II stimulation and was further elevated when combined with E2.

Discussion: These findings support the hypothesis that E2 exerts antihypertrophic effects, potentially by enhancing VCAM-1 expression in cardiomyocytes. Future studies involving ER α /VCAM-1 silencing and sex-specific cardiomyocyte cultures will help clarify the mechanistic role of this pathway in estrogen-mediated cardioprotection.

Acknowledgment: FONDAP 1523A0008 and FONDECYT 1240443.

P6. Royal jelly-derived extracellular vesicles modulate microglial nanomechanics. Pablo Berrios¹ (pablo.berrioss91@gmail.com), Gabriela Zavala², Christina Schuh², Sebastián Aguayo^{1,3}.

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Introduction: Microglia undergo profound mechanical and functional changes upon activation, contributing to neuroinflammation; however, these mechanobiological alterations remain strongly understudied. Honeybee-derived Royal Jelly Extracellular Vesicles (RJEVs) have demonstrated remarkable anti-inflammatory properties, but their impact on microglial cellular nanomechanics and uptake mechanisms remains unclear. In this study, we used atomic force microscopy (AFM) based single-cell force mapping to analyze the resulting nanomechanical changes following the activation of human microglia with LPS and the potential effect of RJEV treatment on these mechanobiological parameters.

Materials and Methods: HMC3 microglial cells were seeded in PLL-treated glass coverslips at 7500 cells per cm² and left to adhere for 24h. RJEVs were added at a 1000 EVs per cell ratio for 20 hours, and 1 μ g/ml LPS was added 4 hours before the measurements. An MFP 3D-SA (Asylum Research, USA) was used for all AFM measurements in HBSS buffer at room temperature for no longer than 2 hours to ensure proper cell viability throughout the experimentation. Mechanical measurements were made at a constant velocity of 8 μ m/s, maximum force applied of 5nN, cantilever nominal spring constant of 0.09N/m (TR400PB, Asylum Research, UK), and scan size of 30x30 μ m-90x90 μ m. Young's modulus was obtained by fitting the data with the JKR model in the IGOR 8.04 software.

Results: The Young's modulus, a measure of cell stiffness and elasticity, ranged from a mean of 4502 Pa for the control group to 1920 Pa for LPS-treated. We observed that LPS treatment was associated with decreased cellular Young's modulus, and RJEV treatment counteracted these mechanobiological changes by increasing microglial

stiffness to 7426 Pa.

Discussion: RJEVs ability to reverse the alterations in cellular mechanics in HMC3 after LPS treatment highlights the use of RJEVs as potential therapeutics. Further work is required to determine the exact mechanism involved in this process.

Acknowledgment: FONDECYT #1220804

Sponsored by: Lorena Lobos

P7. TRPM4 modulates focal adhesion-associated Ca²⁺ signals and dynamics. Mariana Brunett¹ (mariana.brunett@ug.uchile.cl), Ian Silva^{1,2}, Diego Maureira¹, Oscar Cerda¹.

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Introduction: Cell migration is essential for numerous physiological and pathological functions. It relies on cytoskeleton remodeling, changes in focal adhesion (FA) structures, and fluctuations in intracellular calcium (Ca²⁺) levels. TRPM4, a FA-localizing, Ca²⁺-activated non-selective monovalent cationic channel promotes disassembly of FA, a process that is triggered by local oscillatory Ca²⁺ signals associated with these structures. We propose that TRPM4 affects FA turnover by regulating these FA-localized Ca²⁺ signals. Interestingly, in this study, we demonstrate that TRPM4 enhances Ca²⁺ signaling at FA induced by Yoda1, an activator of the also FA-localizing mechanosensitive Piezo1 channel. We observed that TRPM4 is involved in the oscillatory frequency of these signals. Furthermore, our data points to a mechanism that is dependent on NCX (Na⁺/Ca²⁺ exchanger) reverse activity, suggesting that NCX may act as the Ca²⁺-permeable entity regulated by TRPM4 in this context. Finally, we observed consistent effects in experiments monitoring FA disassembly/assembly rates. Overall, this research provides new insights into how FA-associated local Ca²⁺ signals are triggered and shaped, while also pointing to the molecular players involved in this process, which is pivotal for deeper understanding of FA dynamics

Materials and Methods: A FA-targeted Ca²⁺ sensor, mCherry-Paxillin-jGCaMP8f, was developed and FBS or Yoda1-elicited Ca²⁺ signals were recorded using TIRF microscopy. The channel TRPM4 was overexpressed or silenced and Ca²⁺ signals were recorded in MEF cells.

Results: The overexpression and silencing of TRPM4 led to an increase and decrease, respectively, in the frequency of Ca²⁺ oscillations and FA disassembly rates induced by Piezo1 activator Yoda1 or FBS. When evaluating the role of NCX in the effect of TRPM4 on oscillations, it was found that the inhibition of the exchanger reversed the effect of the channel.

Discussion: These data suggest that TRPM4 modulates local Ca²⁺ signals at FAs following Piezo1 activation. The dependence on NCX1 highlights a possible mechanistic link between Piezo1, TRPM4, and NCX-mediated Ca²⁺ entry, and FA remodeling during migration.

Acknowledgment: FONDECYT 1240633.

P8. Aptamer blockade of Lactadherin is secreted by triple-negative breast cancer cells inhibit angiogenesis in an *in vivo* breast cancer model. Karen Carrasco-Maure^{1,2,5} (karen.carrasco@ug.uchile.cl), Cristina Mayorga-Lobos^{1,2}, Kevin Nawrath^{1,2}, Francisca Sepúlveda^{1,2,6}, Mauricio González-Olivares^{1,2,3}, María José Montenegro^{1,5}, Alexander Riquelme^{1,4} y Lorena Lobos-González^{1,2}.

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Introduction: Triple-negative breast cancer (TNBC) lacks estrogen, progesterone, and HER2 receptors, limiting treatment primarily to chemotherapy, which is often associated with poor long-term outcomes. Angiogenesis is

critical for tumor progression, and Lactadherin (MFG-E8), a glycoprotein involved in tumor vascular remodeling, is secreted by MDA-MB-231 cells. Lactadherin-enriched extracellular vesicles (EVs) act as mediators of intercellular communication and angiogenesis. The use of aptamers on pro-angiogenic proteins is unknown. This study aimed to evaluate whether aptamer blockade of Lactadherin inhibits angiogenesis and tumor growth *in vivo*.

Materials and Methods: The EVs from MDA-MB-231 cells were purified and characterized. A subcutaneous tumor formation model was established by inoculating MDA-MB-231 spheroids into immunodeficient mice. The experimental groups included untreated tumors (control), tumors stimulated with EVs MDA-MB-231, tumors treated with anti-Lactadherin 2 or 4 aptamers, and tumors treated with a aptamer scramble. Tumor progression was evaluated by measuring volume and mass, while angiogenesis was preliminarily assessed by hematoxylin-eosin staining. Groups were compared using ANOVA Parametric and nonparametric test.

Results: We showed that the anti-Lactadherin 2 aptamer reduced tumor volume ($108 \pm 102 \text{ mm}^3$) compared to controls ($540 \pm 176 \text{ mm}^3$, $p < 0.01$) and tumor mass ($0.145 \pm 0.097 \text{ g}$ vs. $0.539 \pm 0.188 \text{ g}$, $p < 0.05$). Preliminary histological evaluation indicated a decrease in the number of blood vessels in tumors treated with aptamers

Discussion: This study further supports lactadherin as a key mediator of angiogenesis and tumor progression in TNBC. The selective effect of aptamer 2 highlights the importance of specific inhibition of Lactadherin. These findings support Lactadherin as a potential therapeutic target and suggest that aptamer-mediated blockade may prevent tumor growth and angiogenesis.

Acknowledgment: FONDECYT 1211223 and FONDEF ID25I10020

P9. Topical N-Acetylcysteine as a Therapeutic Candidate in RDEB: Reducing Fibroblast Senescence and Accelerating Wound Closure. Evelyn Catalán (ecatalan@uc.cl)^{1,2}, Belén Dianta^{1,2}, Nicole Koplów¹, Paola Tiozzo-Lyon¹, Pilar Morandé², Francis Palisson², Alexander Nyström³ and Ignacia Fuentes^{1,2,4}.

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Introduction: Recessive Dystrophic Epidermolysis Bullosa (RDEB) is a severe genetic skin disorder characterized by chronic wounds and progressive fibrosis. The persistent wound environment in RDEB generates high cellular stress, which may promote the accumulation of senescent fibroblasts. We previously reported that fibroblasts isolated from RDEB chronic wounds exhibit some features of cellular senescence compared with fibroblasts from non-lesional skin of the same patients, suggesting a role for senescent cells in maintaining chronic wound pathology. The aim of this study was to characterize the senescence phenotype of fibroblast populations derived from distinct RDEB cutaneous microenvironments and to evaluate the senolytic potential of N-acetylcysteine (NAC) *in vitro* and *in vivo*.

Materials and Methods: Fibroblasts derived from chronic wounds, acute wounds and, non-lesional skin of RDEB patients, as well as healthy skin, were used to characterize senescence markers including DNA damage foci (53BP1, γ H2A.X), nuclear morphology, and SA- β -gal activity. Cells were treated with 1 mM NAC for up to 6 days. In parallel, a topical NAC-containing gel was applied to one chronic RDEB wound, and wound closure kinetic was monitored over time.

Results: RDEB fibroblasts from chronic wounds displayed multiple senescence features compared with acute and non-lesional fibroblasts. NAC treatment selectively reduced the proportion of senescent cells, evidenced by cell viability, a lower frequency of fibroblasts with enlarged nuclei and reduced SA- β -gal activity. In one RDEB patient/wound, topical NAC application promoted the closure of a chronic wound only a few days after NAC treatment.

Discussion: These findings highlight the dual effect of NAC in the attenuation of fibroblast senescence *in vitro*, and the potential promotion of wound healing in patients with RDEB. Together, this data supports NAC as a promising therapeutic candidate for targeting chronicity and improving wound resolution in RDEB.

Acknowledgements: FONDECYT 1220704 granted to IF.

P10. NUAK1 regulates SREBP1 maturation and activity through the Akt/mTOR axis in colorectal cancer.

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Introduction: Sterol regulatory element-binding protein 1 (SREBP1) is a master regulator of lipid biosynthesis, whose aberrant activation contributes to tumor growth and metabolic reprogramming in cancer. This factor is canonically regulated by the oncogenic signaling pathway PI3K/Akt/mTOR. NUAK1 is a serine/threonine kinase member of the AMPK-related family; it is overexpressed in several cancers and associated with poor prognosis. NUAK1 has been shown to activate Akt and its downstream targets, as well as to interact with components of the mTOR complexes. NUAK1 has been widely associated with metabolism, specifically cholesterol and fatty acid biosynthesis, regulated by SREBP. In this study, we investigate the role of NUAK1 as a modulator of SREBP1 activity through the AKT/mTOR signaling axis.

Material and methods: HA-hNUAK1 was overexpressed in HCT116P53^{-/-} cells cultured in 10%FBS medium, and treated with the specific inhibitors MK2206, Rapamycin, or Torin. 48 hours post-transfection, cells were lysed or fractionated into cytoplasm and nucleus for western blotting. Flow cytometry, RNA extraction, BODIPY-488 dyeing, and immunocytochemistry were also performed 48h post NUAK1 overexpression in the same conditions.

Results: We found that NUAK1 promotes nuclear translocation of SREBP1 and its transcriptional activity in an Akt/mTOR-dependent manner. Additionally, immunofluorescence revealed that it also promotes the accumulation of lipid droplets.

Discussion: Our findings indicate that NUAK1 promotes SREBP1 maturation and activity through the Akt/mTOR axis.

Acknowledgements: FONDECYT N°1241771 and 1201215, VRID DICA N°170/25

P11. Deletion in the triple helix region at the 3' end of MALAT1 in the MCF-7 breast cancer cell line. Aramis

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Introduction: Breast cancer is one of the leading causes of mortality among women. Its development is influenced by genetic, epigenetic, and environmental factors. Long non-coding RNAs (lncRNAs) have emerged as key regulators of processes such as proliferation, migration, and metastasis. Among them, MALAT1 stands out, as its overexpression has been reported in breast cancer. This transcript performs various cellular functions and presents a triple helix structure at its 3' end, which has been proposed as an essential element for its stability. However, the specific role of this region in maintaining transcript levels in cancer cells has not been fully characterized.

Materials and Methods: CRISPR-Cas9 was used to generate a deletion in the triple helix region at the 3' end of the MALAT1 gene in MCF-7 breast cancer cells. Deletion was confirmed by PCR. MALAT1 transcript levels were evaluated using RT-qPCR and digital PCR. Subcellular localization was determined by FISH. Cell proliferation was measured through direct cell counting.

Results: We obtained cells containing deletions in one allele of the MALAT1 gene. The induced deletion caused a decrease in MALAT1 transcript levels, evidenced by qPCR, digital PCR and FISH, when comparing edited clones with the control group. Additionally, a significant decrease in cell proliferation was observed in some edited clones compared to the control.

Discussion: These results indicate that the 3' end of MALAT1 is involved in its cellular levels and its deletion impacts cell proliferation. These findings provide functional evidence of the relevance of this region and open new questions about its role in the pre- or post-transcriptional regulation of MALAT1 in breast cancer.

Acknowledgment: FONDECYT 1240853, FONDECYT 1250955, FONDEQUIP EQM230028

P12. Squid oil (*Dosidicus gigas*) with potential antiproliferative activity against gastric cancer. Yohana

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Introduction: Gastric cancer (GC) is one of the leading causes of cancer-related mortality in Chile and worldwide. Conventional chemotherapy treatments show limited efficacy and high toxicity. Polyunsaturated omega-3 fatty acids (ω -3 PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been widely studied for their roles in regulating cell proliferation, inflammation, and apoptosis. The hepatopancreas oil of jumbo squid (*Dosidicus gigas*), obtained through the SATREPS project, was found to contain a high proportion of EPA and DHA (~42% of total fatty acids).

Materials and Methods: The antiproliferative effect of squid oil was evaluated in gastric cancer AGS cells and normal gastric GES-1 cells, measured through the MTS assay. The cells were stimulated with different concentrations of the jumbo squid oil extract, expressed as DHA concentration, from 15,6 μ M to 2000 μ M of DHA. IC₅₀ values were determined at 24 h and 48 h post-treatment. All experiments were conducted in triplicate, and data are reported as means \pm standard deviations.

Results: Squid oil significantly decreased the viability of AGS tumor cells in a dose- and time-dependent manner, with IC₅₀ values of 609.7 μ M (24 h) and 455.6 μ M (48 h). In contrast, normal gastric GES-1 cells did not show a relevant reduction in viability under the same treatment conditions.

Discussion: These results demonstrate that *Dosidicus gigas* oil exerts a selective antiproliferative effect on gastric cancer cells, without affecting normal gastric cells. Its high ω -3 PUFA composition positions it as a promising candidate for the development of novel adjuvant and nutraceutical therapies for gastric cancer management.

Acknowledgment: SATREPS Project, National Doctoral Scholarship from ANID (Agencia Nacional de Investigación y Desarrollo, Chile), 2025.

Sponsored by: Claudia Quezada.

P13. IL-6/STAT3 Regulates Scavenger Receptor Expression and Oxidized LDL Uptake in the Progression of Prostate Cancer. Durán A (anduran2016@udec.cl), Azocar S., Castillo M, Duprat F., Olivas G., Pampaloni P., Rivas Y., Sanzana J., González-Chavarría I

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Introduction: Castration-resistant prostate cancer (CRPC) represents a major clinical challenge due to its aggressiveness and limited treatment options. Our group has previously demonstrated that oxidized low-density lipoproteins (oxLDL), through scavenger receptors (SCRs), drive biochemical progression of CRPC by enhancing androgen receptor (AR) and AR-V7 expression. Interleukin-6 (IL-6), a cytokine strongly implicated in castration resistance, promotes tumor progression via STAT3 activation. In cardiovascular diseases, IL-6 has been shown to upregulate SCRs; however, its contribution to SCR expression in prostate cancer (PCa) and CRPC through STAT3 activation remains unexplored. This study aimed to evaluate the role of IL-6 in SCR expression, oxLDL endocytosis, and biochemical progression in cellular models of PCa and CRPC.

Materials and Methods: Androgen-sensitive LNCaP cells and CRPC-derived C4-2B cells were used. IL-6 activity was assessed by Western blot analysis of STAT3 phosphorylation (Y705). The expression of LOX-1 and CD36, two key oxLDL receptors, was evaluated at the protein and mRNA levels by Western blot and RT-qPCR, respectively, in the presence or absence of a STAT3 inhibitor. Functional assays measured Dil-oxLDL uptake to determine the impact of IL-6/STAT3 signaling on lipid internalization.

Results: Treatment with IL-6 (10 ng/mL) induced STAT3 phosphorylation and significantly increased LOX-1 expression at both protein and mRNA levels in LNCaP and C4-2B cells. In contrast, co-treatment with a STAT3 inhibitor abrogated IL-6-induced SCR upregulation and reduced basal receptor levels. Functionally, IL-6 stimulation enhanced oxLDL endocytosis in both PCa cell models, an effect dependent on STAT3 activation.

Discussion: Our findings demonstrate that IL-6/STAT3 signaling upregulates SCRs, particularly LOX-1, thereby enhancing oxLDL uptake. This mechanism may contribute to CRPC progression by providing cholesterol for

intratumoral steroidogenesis, enabling tumor cells to synthesize androgens independently. These insights highlight IL-6/STAT3-SCR signaling as a potential therapeutic target in advanced prostate cancer.

Acknowledgment: FONDECYT 1231911

P14. Not show

P15. Development of a CTGF Inhibitor Based on a Novel Protein-Peptide Interface. Jennifer Faundez-Contreras¹⁻³ (jfaundezc2@correo.uss.cl), Raúl Araya-Secchi²⁻³, Tiaren Ruiz², Enrique Brandan¹⁻³.

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Introduction: Fibrotic diseases are a major cause of morbidity and mortality worldwide, characterized by excessive extracellular matrix (ECM) deposition that disrupts tissue architecture and impairs organ function. Connective Tissue Growth Factor (CTGF) is a matricellular protein consistently overexpressed in fibrotic tissues and plays a central role in fibroblast activation and ECM production, making it a compelling therapeutic target. This project focuses on developing a peptide-based CTGF inhibitor, inspired by a naturally occurring interaction between CTGF and an ECM-associated protein. We previously identified a medium-length peptide fragment that significantly inhibits CTGF activity; however, the structural basis for this inhibition had not been fully elucidated.

Methods: We employed AlphaFold2 and AlphaFold-Multimer to generate structural models of CTGF alone and in complex with the peptide. Models were evaluated using confidence metrics and contact maps, followed by 1.2 μ s molecular dynamics simulations to assess stability. Alanine scanning was performed using Rosetta Flex ddG to estimate residue contributions to binding. Experimental validation included co-immunoprecipitation and fluorescence-based binding assays.

Results: The peptide induced conformational compaction of CTGF, stabilizing interdomain hinge regions and decreasing solvent accessibility at proteolytic hotspots. Alanine scanning identified six residues as critical for binding. Experimental assays confirmed the predicted interaction and binding geometry. The peptide interacts across the CTGF structure, with a specific segment anchoring two distinct regions, supporting a dual-binding mechanism.

Discussion: Our findings provide structural and energetic insights into a novel CTGF-peptide interface, supporting the rational design of CTGF inhibitors. These results lay the groundwork for optimizing peptide stability and affinity, paving the way toward effective, selective CTGF-targeted therapeutics.

Acknowledgments: This work is supported by FONDECYT N°1230054, Proyecto CCTE Ciencia y Vida Basal FB210008 and FONDEF ID25I10016, ANID/BECA DOCTORADO NACIONAL 21241714.

P16. Characterization of Rab GTPase Family Genes in *Eucalyptus globulus* under Drought Stress Conditions. Constanza Frois-Meza¹ (cfrois20@alumnos.utralca.cl), A. Carolina Puentes-Romero¹, Paulo Cañete-Salinas², Raúl Herrera¹.

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Introduction: Rab GTPases, members of the GTPase superfamily, are pivotal regulators of vesicular trafficking, controlling vesicle targeting and tethering to specific membranes. In addition to their well-established roles in plant development, they contribute to abiotic stress responses, including woody species such as *Eucalyptus*, a genus of economic and ecological importance. *Eucalyptus* is characterized for its rapid growth and remarkable adaptability to environmental pressures such as drought whose occurrence and impact are being exacerbated by climate change. However, the specific functions of Rab GTPases in woody species under water deficit remain poorly understood.

Materials and Methods: We investigated Rab GTPases in *Eucalyptus* through a combined experimental and *in silico* approach. Eight-month-old *Eucalyptus* spp. seedlings were subjected to 15 days of water withholding, followed by physiological measurements. Then, leaf and stem samples were collected for relative gene expression analysis by qRT-PCR. Genome-wide characterization of *Eucalyptus globulus* Rab GTPases included, (i)

phylogenetic analyses, (ii) gene structure, (iii) conserved motifs, (iv) cis-regulatory elements, and (v) chromosomal mapping.

Results: A total of 21 *Rab* GTPase genes were identified in *Eucalyptus globulus* genome, classified into eight subfamilies (A–H) and mapped to 11 chromosomes, with no members located in chromosome 3. Gene structure analysis revealed one to seven introns, and most encoded proteins exhibited highly conserved motif architectures. Under drought stress, transcriptional profiling demonstrated distinct tissue-specific expression patterns between leaves and stems, suggesting differential functional roles in stress adaptation.

Discussion: The results provide a deeper understanding of the mechanisms underlying drought tolerance in *Eucalyptus*, based from physiological parameters and on the tissue-specific transcriptional responses observed. Furthermore, the relationships between the structure, localization, and function of vesicular transport genes were elucidated, offering a foundation for future studies aimed at identifying their molecular targets and enhancing stress tolerance in trees.

Acknowledgements: ANID-Anillo ATE220043, ANID-FONDECYT postdoctorado N° 3240627 and FONDECYT N°1241579.

P17. Toward a Structural Map of the CTGF/CCN2 Interactome: Predicting Domain–Partner Interactions.

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Introduction: Connective tissue growth factor (CTGF/CCN2) is a multifunctional, cysteine-rich protein of 38 kDa composed of four modular domains. It acts as a central signaling hub, orchestrating key biological processes such as cell proliferation, extracellular matrix remodeling, angiogenesis, and wound healing. In pathological contexts, CTGF has been implicated in fibrotic disorders, cancer progression, and chronic inflammatory diseases, underscoring its value as a potential therapeutic target. Despite decades of research, the absence of a resolved three-dimensional structure limits our ability to fully understand how CTGF engages with its wide array of partners. Current evidence suggests that its versatility is mediated by domain–domain cooperativity and selective binding to growth factors, integrins, and regulatory proteins. However, the structural basis of these interactions remains poorly defined, and a comprehensive map of the CTGF interactome has yet to be established.

Methods: To address this gap, we performed structural modeling of CTGF protein–protein interactions using AlphaFold2-multimer and Boltz2. These state-of-the-art computational approaches allow prediction of multimeric complexes and conformational ensembles, offering insight into dynamic binding mechanisms. Predicted structures were systematically compared with reported biochemical and cellular data to evaluate their reliability. This integrative approach not only validates the accuracy of the models but also highlights discrepancies where computational predictions suggest novel structural features not yet experimentally described.

Results: Initial results revealed potential binding sites for TGF- β and integrin $\alpha 5\beta 1$, two critical partners in processes of fibrosis and cell adhesion. These predictions align with previously reported experimental findings, while simultaneously providing new mechanistic insights into how CTGF may stabilize or regulate these interactions at the molecular level. Furthermore, our models suggest the existence of additional, previously unrecognized interaction hotspots that may contribute to the broad functional repertoire of CTGF. Ongoing analyses are extending this framework to other candidate partners, with the goal of generating the first integrative structural map of the CTGF interactome.

Discussion: By uncovering the structural determinants of CTGF binding versatility, this work advances our understanding of how a single modular protein can regulate diverse physiological and pathological pathways. The identification of interaction hotspots paves the way for the rational design of antibodies, peptides, and small molecules capable of selectively modulating CTGF activity. Ultimately, these findings contribute to establishing a structural foundation that may guide future therapeutic strategies for conditions such as fibrosis and cancer, where CTGF plays a central pathogenic role.

Acknowledgments: This work is supported by FONDECYT N°1231164, Proyecto CCTE Ciencia y Vida Basal FB210008 and FONDEF ID25I10016.

P18. Photomodulation of cyanobacterial FNR function defines light-controlled mechanism of catalytic plasticity supporting photosynthetic acclimation to variable light. Valentina Garrido-Espinoza (vagarrido18@alumnos.otalca.cl)¹, Pablo Galaz-Davison¹, Cristian Tirapegui², Gabriel Vallejos³, Pedro General⁴, Horacio Poblete¹, Ricardo A. Zamora⁵, Alejandra Herrera-Morandé⁶.

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Introduction: Photosynthetic organisms adapt to daily light fluctuations. In cyanobacteria, changes in light intensity regulate the switch from linear (LEF) to cyclic electron flow (CEF). A key difference between LEF and CEF is the production of NADPH by ferredoxin–NADP⁺ reductase (FNR), which receives electrons from ferredoxin (Fd). High light intensities reduce the FNR–Fd interaction and redirect electron flow toward CEF. To date, a direct influence of light on FNR function or its regulatory role has not been demonstrated.

Methods: We investigated the photomodulation of cyanobacterial FNR function under red and blue light, focusing on its interaction with NADP(H) and NAD(H). Kinetic parameters for both cofactors were determined under dark, red, and blue light conditions. Dissociation constants (K_d) for FNR:NADP⁺ and FNR:NAD⁺ were measured under identical illumination conditions. Rosetta-based perturbation modeling and molecular docking were used to assess structural configurations compatible with experimental observations.

Results: Catalytic efficiency toward NADP(H) was similar under blue light and darkness but decreased under red light. Conversely, catalytic efficiency toward NAD(H) increased under blue light and dropped under red light. Affinity for NADP⁺ was highest in the dark and decreased under blue and red light. Under dark conditions, binding data fit a one-site binding model, whereas under blue and red light, data fit a two-site binding model. NAD⁺ binding was observed exclusively under blue light and was described by a two-site binding model. Structural perturbations introduced in silico at residue Y235 caused structural diversification toward catalytically-preformed configurations, confirming that local rearrangements could significantly impact catalysis and binding.

Discussion: The present work suggests that photomodulation of FNR function may represent a novel regulatory mechanism driven by its light-dependent catalytic plasticity. These findings support a direct role for FNR in photosynthetic acclimation, highlighting a previously unrecognized photoregulatory function.

Acknowledgment: FONDECYT 1240759, FONDECYT 11250892

P19. DEAD-box RNA helicase DDX3X is recruited to the Zika virus replication compartment to promote viral RNA translation in human microglia. Alonso González-Poblete^{1,2,3} (alonsogonzalez@ug.uchile.cl), Tomás Hernández-Díaz^{1,2,3}, Sebastián Giraldo-Ocampo^{1,2,3}, Cecilia Rojas-Fuentes^{1,2,3}, Aarón Oyarzún-Arrau^{1,2,3}, Fernando Carrasco-Galvez^{1,2,3}, Mónica Acevedo-Acevedo^{1,2,3}, Aracelly Gaete-Argel^{1,2,3}, Barbara Rojas-Araya^{2,4}, Marcelo López-Lastra^{2,4}, Matías Zúñiga-Bustos⁵, Tommaso Cupido⁶, Fernando Valiente-Echeverría^{1,2,3} and Ricardo Soto-Rifo^{1,2,3}.

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Introduction: Zika virus (ZIKV) is a re-emergent mosquito-borne virus. ZIKV can infect different tissues and cell types, including microglia in the central nervous system. Microglia infection has been correlated with neuroinflammation and the development of neurological disorders. Although cells trigger an antiviral state in response to infection, flaviviruses such as ZIKV and dengue virus (DENV) can exploit components of the machinery involved in the antiviral response, such as the DEAD-box RNA helicase DDX3X, to promote viral replication. In this work, we explored the involvement of DDX3X during ZIKV replication in a human microglia cell line.

Materials and methods: ZIKV and DENV WT were used to infect the C20 human microglia cell line. Confocal microscopy was used to locate cellular and viral components. Using knockdown of DDX3X and a ZIKV-based reporter RNA, we investigated the role of DDX3X in ZIKV replication.

Results: Our data show that both ZIKV and DENV recruit DDX3X to the viral replication compartment in human microglia. However, silencing of DDX3X only affected ZIKV replication, resulting in a decrease of viral protein levels and viral titer, with a marked increase in cytoplasmic levels of viral RNA (vRNA). Confocal microscopy experiments suggest that DDX3X can interact with vRNA, and by using a ZIKV-derived reporter RNA in DDX3X knockdown cells, we show that the host RNA helicase participates in vRNA translation.

Discussion: Our results indicate that DDX3X acts as a ZIKV-specific proviral factor in human microglia to promote viral protein synthesis.

Acknowledgement: ANID-FONDECYT Program, grants N° 1230102 to R.S.R.; N° 1251218 to F.V.E.; N° 1210736 to M.L.-L. and N° 11230976 to M.Z.-B. ANID-ICM, ICN 2021_045 to R.S.R., F.V.E. and M.L.L., Anillo Grant ATE220016 to R.S.R. and F.V.E.

P20. Understanding the role of FchNAC1 in cell wall disassembly associated to softening of *Fragaria chiloensis* fruit. Macarena Zamorano-Curaqueo (mzamorano@utalca.cl), Joselin Guajardo, Raúl Herrera, M. Alejandra Moya-León.

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Introduction: The transcription factor (TF) FchNAC1 is member of the NAC family (for NAM, ATAF1/2 and CUC2), exclusive to plants, and proposed to be involved in the regulation of fruit ripening. FchNAC1 was identified and described as a trans-activator of the promoter sequence of *FchPL* (Carrasco-Orellana et al., 2018), a gene that participates in cell wall disassembly during ripening of *F. chiloensis* fruit. The aim of this work is to clarify the role of FchNAC1 on the transcriptional regulation of genes coding for enzymes involved in cell wall disassembly through overexpression and gene silencing strategies in *Fragaria chiloensis* fruit.

Materials and Methods: *F. chiloensis* fruit at C2 stage were transiently transformed with *FchNAC1* silencing (RNAi) or overexpression constructs, and used to evaluate the expression of genes involved in cell wall disassembly by means of RT-qPCR. In addition, the transactivation activity of FchNAC1 on the promoter sequences of *FchXTH1* and *FchRGL* was analyzed through luciferase dual assays.

Results: An increase in the relative expression of *FchXTH1*, *FchRGL* and *FchPG* was observed in *FchNAC1* overexpressing fruits, meanwhile a repression in *FchXTH1*, *FchRGL* and *FchPG* was quantified in *FchNAC1* silencing fruit. Non-significant changes in expression were recorded for *FchEXP5* and *FchPL* either by the overexpression or silence of *FchNAC1*. On the other hand, FchNAC1 transactivates the promoter sequence of *FchRGL* that contains *cis* elements recognized by NAC TFs.

Discussion: These results show that FchNAC1 plays a relevant role in the transcriptional regulation of genes that participate in cell wall disassembly in *Fragaria chiloensis* fruit.

Acknowledgment: FONDECYT 1251538 and ANILLO ATE220043.

P21. Study of the impact of the polypyrimidine tract-binding protein on the translational activity of the IRES elements present in the sense and antisense mRNAs of HTLV-1. Daniela Guzmán-Correa (Daniela.guzman@uc.cl), Marcelo López-Lastra.

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Introduction: The capped sense mRNA of the human T-cell leukemia virus type 1 (HTLV-1) harbors an internal ribosome entry site (IRES) and can initiate translation via a cap-dependent or IRES-dependent mechanism. HTLV-1 also expresses two antisense transcripts, spliced and unspliced versions, which encode for two isoforms of the HBZ protein, associated with the virus's pathogenicity. The spliced HBZ mRNA (*shbz*) harbors an IRES. IRES activity is modulated (stimulated or decreased) by RNA-binding proteins (RBPs), termed IRES trans-acting factors (ITAFs). The polypyrimidine tract-binding protein (PTB), a well-characterized ITAF for IRESs present in the mRNAs of several viruses, binds to the HTLV-1 RNA in the region that harbors its IRES activity. Three isoforms of PTB exist (1, 2, and 4), which stimulate the activity of various IRESs differentially. This study evaluated the impact of PTB1 and PTB4 on the HTLV-1 IRES and sHBZ IRES activity.

Materials and Methods: PTB overexpression and siRNA-mediated knockdown assays were performed in HEK293T cells expressing a Renilla/Firefly luciferase bicistronic RNA, harboring the HTLV-1 IRES or sHBZ IRES in their intercistronic region.

Results: Neither PTB1 nor PTB4 overexpression significantly impacts the HTLV-1 IRES activity, although high amounts of PTB levels induced changes in sHBZ IRES activity. Endogenous PTB partial knockdown did not show significant changes in the HTLV-1 IRES or sHBZ IRES activity.

Discussion: Together, these findings indicate that PTB is not an ITAF for the HTLV-1 IRES or sHBZ IRES. This finding challenges the prevailing notion that all RBPs associated with the RNA region that harbors an IRES function as an ITAF.

Acknowledgment: The work was supported by grants FONDECYT 1250350 and the Iniciativa Científica Milenio (ICM), IMII (ICM-ANID, ICN2021_045).

P22. Analysis of HDAC6-STAT3-PP2Ac interaction in colorectal cancer cell lines. M. Estrella Armijo, Yanitza Gutiérrez (ygutierrezm@magister.ucsc.cl), and Matías I. Hepp.

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Introduction: Histone Deacetylase 6 (HDAC6) is a protein related to various cancer-promoted mechanisms through regulation of STAT3, a transcriptional factor that affects the expression of different oncogenes and tumor suppressors. In melanoma, HDAC6 interacts with and upregulates STAT3, increasing levels of p-STAT3 (Tyr705) and its translocation to the nucleus. HDAC6 also interacts with PP2Ab, a regulatory subunit of the phosphatase PP2A and a negative regulator of STAT3. Accordingly, in HT29 cells (a colorectal cancer cell line), we have observed that HDAC6 also interacts with STAT3. Nevertheless, the effects of HDAC6 in the STAT3 pathway in CRC is not well-known. The aim of this work was to further analysis the HDAC6-STAT3 axis to provide insight into HDAC6 signaling mechanisms and its potential as therapeutic target in CRC.

Materials and Methods: HCT116 and HT29 cell lines were overexpressed with HDAC6-Flag and treated with IL6 or Nexturastat A. Whole cell lysate was then used for immunoprecipitation to analyze the HDAC6-STAT3 interaction under different treatments and western blot to confirm activation or inhibition of the STAT3 pathway.

Results: In colon cancer cell lines, HDAC6-Flag interacts with STAT3 and with PP2Ac, the catalytic subunit of PP2A. The interaction between STAT3 and HDAC6-flag increased when cells are treated with IL-6, an activator of the STAT3 pathway, and decreases when cells are treated with NextA, an HDAC6 inhibitor that affects the STAT3 pathway.

Discussion: Together, these experiments showed that HDAC6-STAT3-PP2A axis exist in CRC, which provide insight into HDAC6 signaling mechanisms and its potential as therapeutic target in CRC.

Acknowledgment: FONDECYT de Postdoctorado n°3240256.

P23. FchDOF2 as a transcriptional regulator during softening of *Fragaria chiloensis* fruit. Aline Jeria-Orellana^{1,2} (ajeria20@alumnos.utralca.cl), Macarena Zamorano-Curaqueo¹, Joselin Guajardo¹, Raúl Herrera¹, María Alejandra Moya-León¹.

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Introduction: *Fragaria chiloensis* is a native Chilean species whose fruit exhibits non-climacteric ripening pattern and undergoes rapid softening, limiting its postharvest life. Softening involves extensive cell wall remodeling, regulated by hormonal signals such as abscisic acid (ABA) and auxins, and coordinated at transcriptional level by several transcription factors (TF). DOF (DNA-binding with one finger) TFs have been implicated in the regulation of gene expression in various plant species, however, the role in *F. chiloensis* remains poorly understood. The aim of this work was to characterize FchDOF2 as a transcriptional regulator during fruit softening.

Materials and Methods: *FchDOF2* coding sequence was identified, and its expression in developing-ripening fruit and vegetative tissues was analyzed by qPCR. Hormonal treatments (ABA, auxins) were applied to immature fruit (C2 stage). Subcellular localization and Bimolecular-Fluorescence-Complementation (BiFC) assays were performed to confirm nuclear localization and to define TF interaction partners.

Results: *FchDOF2* encodes a 293-amino-acid protein with typical DOF features and a nuclear localization signal. Its expression in the nucleus was confirmed. The expression is fruit-specific, with a peak of expression at C3–C4 ripening stages. Auxins triggered a rapid accumulation of *FchDOF2* transcripts (4–6 h), while ABA induced a latter expression rise (6–24 h). BiFC assays showed that FchDOF2 interacts with FchMYB1 and FchNAC1, both TFs linked to cell wall disassembly.

Discussion: These findings suggest that FchDOF2 integrates hormonal signals and interacts with ripening-associated TFs, positioning it as a potential regulatory hub in softening-related gene networks in *F. chiloensis*.

Acknowledgement: FONDECYT 1251538, and to Universidad de Talca for “Guillermo Blanco” Fellowship.

P24. Unique paradigms found in fungal sRNA processing and function. Nathan R. Johnson^{1,2} (nathan.johnson@umayor.cl), Lorena Melet³, Fabian Gonzalez¹, Paulina Masse¹, Barbara Bernal¹.

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Introduction: Small RNAs (sRNAs) are an ancient and important system of genomic regulation found throughout eukaryotic organisms. Numerous classes sRNAs have been described over decades of research, drawing clear links between genomic phenotypes and functional roles of sRNA genes. However, the majority of our knowledge is focused on plant and animal data, with comparatively little known in fungi. For example, miRNAs are perhaps the best described class of sRNA and, despite many publications in fungi, a universal description of this class remains elusive. This is in-part due to a lack of meta-analyses on a clade-wide scale in fungi.

Materials and Methods: To answer these fundamental questions, we mined public repositories for sRNA-data, identifying over 1,200 libraries across 86 species. We proceeded to process and annotate all sRNA genes in all conditions using our software pipeline optimized for fungal sRNAs. We then merged condition-level annotations into metaloci and filtering these for only experimentally replicated loci

Results: We find less than 2,000 confident sRNA-genes across the 33 fungi with multiple projects. These are dramatically fewer loci than we generally find in other eukaryotes. Furthermore, these are biased for an even smaller subset of organisms, showing that sRNA roles are non-uniform across fungal species. We also find that a remarkably high proportion of these loci come from mRNA and structural RNA regions in the genome, linking sRNA processing to other transcripts, and supporting a cis-regulatory role for sRNAs in fungi. Examining hairpin-derived sRNAs shows that fungal miRNAs rare and less-precisely processed than in other eukaryotes, with intriguing signs of miRNA-related classes novel to fungi.

Discussion: Using these data, we have built a community resource for fungal sRNAs, helping to define broad characteristics of fungal sRNAs. This work shows novel insight into this underexplored kingdom, pointing to unique paradigms, processes, and even classes of sRNAs for fungi.

Acknowledgment: FONDECYT 11220727, Millennium Institute for Integrative Biology (iBio)

P25. Influence of biofilm *Streptococcus mutans*-derived extracellular vesicles on the adhesion and growth capacity of *Streptococcus sanguinis*. Camila Leiva-Sabadini¹ (caleiva2@uc.cl), Céline M. Lévesque², Laurent Bozec², Christina Schuh³, and Sebastian Aguayo^{1,4}.

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Introduction: *Streptococcus mutans*, a cariogenic bacterium, produces bacterial extracellular vesicles (bEVs) that mediate cellular communication. The growth effect of bEVs in the antagonistic bacteria *Streptococcus sanguinis* has been investigated; however, the impact of biofilm-derived bEVs and the adhesion changes remains unknown. In this research, we investigated the impact of biofilm-derived bEVs on *S. sanguinis* growth and quantified single-cell adhesions using atomic force microscopy-based single-cell force spectroscopy (AFM-SCFS).

Material and methods: *S. mutans* UA159 was grown as biofilms on collagen substrates (native and glycated) and planktonic state using BHI medium, at 37 °C for 24 hours. Subsequently, bEVs were obtained using filtration and ultracentrifugation. For growth curves, *S. sanguinis* SK36 was incubated with 5×10^9 particles/ml of bEVs for 22 hours at 37°C. For SCFS, *S. sanguinis* was incubated with bEVs at 37 °C and 5% CO₂ and immobilized onto 0.1% w/v poly-L-lysine-functionalized AFM cantilevers. For each treatment, 400 force curves were generated to determine maximal adhesion force, single unbinding rupture lengths, rupture forces, and contour length (n=3 per condition, p<0.05).

Results: *S. sanguinis* treated with biofilm-derived *S. mutans* bEVs generated a reduction in growth and adhesion to collagen substrates. A more noticeable difference was observed in the treatment with bEVs from glycated collagen surfaces, while treatment with planktonic bEVs increased *S. sanguinis* adhesion, as reflected by the resulting maximum adhesion forces, rupture and contour lengths, and single unbinding forces at 5-second contact times.

Discussion: *S. mutans* bEVs from the biofilm state reduce *S. sanguinis* growth and adhesion compared to bEVs isolated from the planktonic state. This demonstrates the differential modulatory effect of *S. mutans* bEVs as a function of biofilm formation, supporting our prior findings on bEVs dynamics and protein cargo changes because of environmental variations.

Acknowledgment: Fondecyt Regular #1220804, ANID Scholarship #21220799, and Imaging Facility, Faculty of Dentistry UofT, Canada.

Sponsored by: Lorena Lobos

P26. Dual effect of poly(dA:dT) sequences on nucleosome sliding activity of the RSC complex. [Leonor Leiva P. \(leleiva2021@udec.cl\)](mailto:leleiva2021@udec.cl), Fernanda Raiqueo, José L. Gutiérrez.

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Introduction: Chromatin is a dynamic structure whose fundamental unit, the nucleosome, can be reorganized by remodeling complexes like RSC, which are essential for the regulation of gene expression in *S. cerevisiae*. Previous studies have shown that poly(dA:dT) tracts stimulate RSC activity and that this complex mobilizes nucleosome cores away from these sequences. Interestingly, under a design that forces RSC to mobilize a tract into the nucleosome core, using probes where tracts flank a nucleosome core, DNA sliding is impeded, suggesting that they interfere when positioned at a specific nucleosomal region. This work aims to further analyze this property of poly(dA:dT) tract and to determine the nucleosomal region where these sequences produce a block to RSC-mediated sliding.

Materials and Methods: DNA probes containing a nucleosome positioning sequence and poly(dA:dT) tracts at different positions were generated by PCR from plasmids specifically generated for each design. The probes were then reconstituted into mononucleosomes and were used to analyze sliding activity of RSC1 and RSC2 complexes. Time-course analyses were used to determine the extent of sliding hindering and tracts located closer to the nucleosome dyad were used to define the position where sliding stalls.

Results: An initial stimulation of sliding activity was observed in probes containing tracts on both sides of the nucleosome core, followed by a block given by only one tract orientation. The extent of sliding was more restricted in probes harboring tracts closer to the nucleosome dyad. The effects were similar for both RSC complexes.

Discussion: Our results confirm that poly(dA:dT) have the property of hindering RSC's sliding activity when reaching an internal region of the nucleosome. Our findings will allow to study this property of poly(dA:dT) tracts or other sequences in higher eukaryotes, contributing to uncover the role of these sequences in chromatin dynamics and gene regulation.

Acknowledgment: Universidad de Concepción VRID-Investigación 2023000737INV.

P27. Study of new formulations for the control of plant pathogens: physicochemical properties and their effects in *Botrytis cinerea* proliferation. Ligia Llovera^{1,3} (ljlloveras83@gmail.com), Ignacio Moreno², Mauricio Osorio¹, Lautaro Torga³, Andrés F. Olea¹.

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Introduction: *Botrytis cinerea* is a necrotrophic fungus that generates important economic losses in Chile. To control this pathogen, our laboratory has designed and synthesized new chemical compounds and delivery systems. These strategies have been tested successfully *in-vitro* and *in-planta* assays controlling the proliferation of *B. cinerea*, but the mechanism involved is not known. In this work, we aim to investigate the effects of different formulations and their interaction with the plant leaf surface in the establishment of pathogen resistance.

Materials and Methods: Several compounds and delivery methods were assayed in agar plates and plant leaves. Physical properties of the formulations, such as superficial tension and contact angle over leaves were measured, as well as total amount of organic acids generated after the application of the formulations over the plant.

Results: Our formulation successfully controls *B. cinerea* in *in-vitro* assays and on plant leaves. In addition, plants treated with commercial fungicides as well plants treated with our formulations showed an increased amount of salicylate and/or methyl-jasmonate.

Discussion: Our results suggest that the effectiveness of a fungicide formulation is related to its capacity to increase its surface contact over the target tissue. Approaches to increase the area of contact will be discussed. We envision that to generate a more efficient compound or formulation for pathogen control; it is also necessary to consider its effect on the systemic acquired resistance of the plant.

Acknowledgment: FONDECYT 1221583

Sponsored by: Adrián Moreno.

P28. 17 β -estradiol promotes pulmonary arterial smooth muscle cell migration via senescence induction. Camila López-Torres^{1,2} (camila.lopez.t@ug.uchile.cl), Francisco Sigcho^{2,3}, Jaime A. Riquelme^{1,3}, Valentina Parra^{2,3,4}.

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Introduction: Pulmonary arterial hypertension (PAH) is a disease characterized by vascular remodeling driven by phenotypic and metabolic changes in pulmonary arterial smooth muscle cells (PASMCs). A key mechanism contributing to this process is the accumulation of senescent cells, which promote remodeling through the secretion of diverse molecules. Elevated levels of 17 β -estradiol have been associated with increased severity of PAH by promoting the pathological phenotypic switch of PASMCs; however, the mechanisms underlying this effect remain unexplored. Although 17 β -estradiol has been reported to induce senescence in other cell types, it is still unclear whether it triggers a similar response in human PASMCs. Therefore, we aimed to evaluate whether 17 β -estradiol induces senescence in PASMCs and whether this effect impacts functional properties such as cell migration.

Materials and methods: Human PSMCs were treated with 17 β -estradiol (100 nM, 72 h). Senescence was evaluated through β -galactosidase activity (staining kit); protein levels of p16, p21, and phosphorylated retinoblastoma (pRb) (Western blot); mRNA expression of p16 and p21 (qRT-PCR); and DNA damage (γ H2AX) together with cell morphology (immunofluorescence). Cell migration was assessed by wound healing assay, with or without the senolytic navitoclax (1 μ M, 24 h).

Results: Treatment with 17 β -estradiol increased β -galactosidase staining and reduced pRb protein levels, while no changes were detected in p16 or p21 protein and mRNA expression. Immunofluorescence analysis of γ H2AX revealed an increased number of nuclear foci, along with enlarged cell perimeter and area. Furthermore, 17 β -estradiol enhanced cell migration in the wound healing assay, an effect that was prevented by co-treatment with the senolytic navitoclax.

Discussion: Taken together, these results suggest that 17 β -estradiol induces senescence in a subpopulation of hPSMCs and promotes cell migration, an effect that appears to be dependent on 17 β -estradiol-induced senescence.

Acknowledgments: This project is funded by ANID FONDECYT 1231576 (JR) and 1230195 (VP); and FONDAP 15130011 (VP, JR).

P29. Structural and functional characterization of C-terminal region of DSUP. Elías Manríquez B.¹(elias.manriquez@ug.uchile.cl), Martín Uriz² and Exequiel Medina G.^{1,2},

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Introduction: The Damage Suppressor Protein (DSUP), exclusive from the tardigrade *Ramazzottius varieornatus*, has been reported as a shield that protects DNA from ionizing radiation, and with the ability to aggregate into molecular condensates. In terms of structure and function, it has been described that is the C-terminal region of the protein (CDSUP) that has the capability to interact nonspecifically with DNA. However, the lack of a biophysical approach to study this protein obscures the relationship between structure and function. The purpose of this study is to describe the structural and DNA-binding properties of the protein and prove the ability to form molecular condensates and the role of the DNA on this process.

Materials and Methods: Circular dichroism (CD) was used to determine the secondary structure composition, and single molecule Fluorescence Anisotropy (smFA) was employed for two single cysteine mutants labelled with BODIPY FL for CDSUP, probing the anisotropy in absence and presence of DNA. On the other hand, condensates assays were performed by optical density measurements and evaluating the effects of different salt and DNA concentrations.

Results: CD corroborated the absence of secondary structure, whereas smFA clearly showed at local level in both mutants a low anisotropy, which is expected for a disordered protein. However, with the addition of DNA a small population appeared at high values of anisotropy, suggesting that DNA stabilizes the flexibility of CDSUP. On the other hand, turbidity assays showed that CDSUP is able to form molecular condensates at very low ionic strength, being its ligand a strong inhibitor of this process.

Discussion: Experiments demonstrate that CDSUP has the biophysical properties of an IDP that also can form condensate states. However, the presence of DNA has significant effects in this set of properties, shedding light on a possible mechanism of biological regulation.

Acknowledgment: FONDECYT 1251879

P30. Calcium and mitophagy on PDGF-BB-induced vascular smooth muscle cell dedifferentiation. Javiera Martínez Bilbao^{1,2} (ivanea.martinez@gmail.com), Ignacio Norambuena-Soto^{1,3}, Fernanda Sanhueza-Olivares^{1,4}, Tamara González-Rivera¹, Francisca Valenzuela-Arce¹, Andrea Mella-Torres¹ & Mario Chiong Lay¹.

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Introduction: Vascular smooth muscle cells (VSMC) contractile function regulates vascular tone and blood pressure. These cells change phenotypes in response to ambient stimuli. Vascular damage triggers VSMC to dedifferentiate from a contractile to a synthetic and proliferative phenotype. Deregulated dedifferentiation constitutes a common event for cardiovascular diseases. Platelet-derived growth factor-BB (PDGF-BB) is a molecule that plays a significant role in VSMC dedifferentiation, and it induces autophagy, changes in mitochondrial function, and mitochondrial fission. However, other aspects that may occur due to these events such as mitophagy or calcium deregulation remains understudied. In this work, we aim to elucidate the effects of PDGF-BB on PINK-1/Parkin and BNIP-3-dependent mitophagy and IP3R-dependent calcium signaling in VSMCs.

Materials and methods: Dedifferentiation of vascular smooth muscle cells of the A7r5 cell line was stimulated with PDGF-BB during 24 and 48 hours. Dedifferentiation was assessed measuring α -SMA, calponin, SM22, GAPDH by western blot and migration by wound-closure assay and transwell. Parkin, PINK-1 and p62 levels were measured by western blot in total cell extracts. PINK-1 and BNIP-3 were also measured in mitochondrial extracts. Contacts between ER-mitochondria were observed by immunofluorescence. IP3R1 and HERPUD1 levels were evaluated by western blot. Data were analyzed using Student t-test or Mann-Whitney test. Results are presented as the mean \pm S.E.M.

Results: PDGF-BB induced A7r5 dedifferentiation assessed by a decrease of α -SMA, calponin and SM22. PDGF-BB induced mitophagy associated with an increase in PINK-1/Parking and BNIP3 protein levels. However, PINK-1 silencing did not prevent PDGF-BB-induced mitophagy. On the other hand, PDGF-BB reduced IP3R1 protein levels, probably through an HERPUD1 dependent mechanism.

Discussion: Further work is required to verify BNIP-3-dependent mitophagy and HERPUD1-dependent IP3R degradation are involved in VSMC dedifferentiation induced by PDGF-BB.

Acknowledgments: FONDECYT 1220392, FONDAP 1523A0008, and Beca ANID 21232137.

P31. Identification, structural modeling and functional analysis of four alcohol acyltransferases from *Fragaria chiloensis* involved in ester biosynthesis. Medina-Farías N. (nmedina20@alumnos.otalca.cl), Guajardo J., Valenzuela-Riffo F., Herrera R., Moya-León M.A.

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Introduction: Fruit aroma is a quality attribute highly appreciated by consumers that develops during fruit ripening. Esters are main aroma-active compounds, conferring fruity and floral notes. Esters are synthesized by alcohol acyltransferase (AAT) enzymes through esterification reactions between acyl-CoAs and alcohols. *Fragaria chiloensis* is a non-climacteric fruit characterized by its attractive fruity aroma evolved during ripening, however a set of other esters are produced at early development stages conferring other biological functions. Therefore, the objective of this study is to identify and characterize FchAATs that could be responsible for the biosynthesis of such esters.

Materials and Methods: The search for *FchAAT* genes was conducted on a *F. chiloensis* fruit transcriptome database. The analysis of sequences was performed by classical bioinformatic tools to confirm catalytic domains. Structural protein models were obtained through homology modelling and after minimization were used in molecular docking analysis with various substrate combinations (acyl-CoAs and alcohols). Expression profiles were obtained by RT-qPCR.

Results: Four *AAT* genes were selected based on the presence of the two conserved motifs characteristic of this family: HxxxD and DFGWG. Two genes are expressed at early development stages meanwhile the remaining two at the ripe stage. Three-dimensional models were built showing globular conformations, in which the catalytic motifs were oriented towards the solvent channels. The pockets for interaction with acyl-CoAs differed in volume and area among the FchAATs. The pockets size and shape for the second ligands, the alcohols, also varied among the FchAATs. The affinity energies determined for acyl-CoAs interaction diverged among protein models, however display similar values for the alcohols.

Discussion: This study demonstrates that several FchAATs are expressed along the entire development and ripening of *F. chiloensis* fruit. The divergent properties of FchAATs identified through bioinformatic tools could explain the synthesis of esters throughout the whole life fruit period.

Acknowledgment: ANILLO ACT210025 and FONDECYT 1251538.

P32. Senescence in rat aorta vascular smooth muscle cells induced by iNOS. Mella-Torres A. (andrea.mella@ug.uchile.cl), Silva D., López E., Lavandero S., Chiong M. Advanced Center for Chronic Disease (ACCDiS), Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile.

Introduction: In reactive oxygen species (ROS)-mediated senescence, oxidative stress causes damaged molecules accumulation, increasing the incidence of cardiovascular diseases. Chronic exposure to NO produced by iNOS during inflammation has been shown to induce senescence in several immortalized cell lines. However, there is few evidence linking iNOS directly to the generation of senescence in vascular smooth muscle cells (VSMCs). The aim of this work was to evaluate the effect of iNOS overexpression on senescence in rat aorta VSMCs.

Materials and Methods: HEK293 cells were used to amplify adeno GFP (AdGFP), and adeno iNOS (AdiNOS), and they were purified by an Adenovirus purification kit (Norgen). A7r5 cells, passages 5-8, were transduced or not with AdiNOS for 72 hours. Adenoviral transduction in A7r5 cells was confirmed by inverted epifluorescence microscope on days 3, 5 and 7 by ZsGreen1(AdiNOS) or GFP reporter fluorescence. The efficiency of adiNOS transduction was determined by immunofluorescence, resulting in 58% at 7 days. The expression of iNOS were evaluated by qPCR (n=3) and western blot (n=5). The cytotoxicity of overexpression was evaluated by measuring cleaved Caspase-3 (n=3). Senescence was assessed by γ -H2AX (n=3), p21 (n=3) and ki-67 (n=3) by immunofluorescence, and phospho-Retinoblastoma (pRb) (n=3) by western blot. Results are presented as mean \pm S.E.M. The n corresponds to independent experiments. Significance was determined by two-way ANOVA followed with Tukey's test ($p < 0.05$) using GraphPad Prism.

Results: Overexpression of iNOS did not induce apoptosis but increased significantly mean γ H2AX foci per nuclei at 5 days post-transduction, while p21 and ki-67 increase at 7 days post-transduction. pRb levels did not show significant changes.

Discussion: Our results suggest that overexpression of iNOS induces senescence in VSMCs *in vitro*, as evidenced by the results obtained in the evaluation of the γ -H2AX markers, p21 and ki-67. However, further work is required to confirm these results.

Acknowledgment: This work was supported by Fondecyt 1220392, FONDAP 1523A0008 and Beca de Doctorado Nacional 21202230, ANID, Chile.

P33. Monitoring DNA–Peptide Complex Assembly in Real Time with Magnetic Tweezers. Michelle Mendoza Becerra¹ (Michelle.Mendoza@mayor.cl), Paula Santana Sepúlveda², Romina Muñoz Buzeta³, Nathalie Casanova Morales⁴, Vicente Salinas Barrera⁵, Jaime Andrés Rivas Pardo¹.

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Introduction: Specific interactions between macromolecules, which can dynamically associate and dissociate, are essential for the regulation of biological functions. A prominent example is the interactions between DNA and proteins, where transcription factors or restriction enzymes recognize particular sequences, enabling precise binding and the control of fundamental cellular processes. Peptides, due to their small size and high affinity for specific DNA sequences, have become valuable tools to study these interactions at the molecular level. Here, we have implemented an assay to track in real-time the formation of a peptide-DNA complex through the use of magnetic tweezers (MT). Specifically, we use the peptide ssIL-8 α , a short peptide made of 17 residues with antimicrobial activity and recently related to hypothetical DNA binding.

Materials and Methods: Double-stranded DNA was obtained by conventional PCR from the pFN18a vector, incorporating an amino group at one end and biotin at the other through modified primers. For DNA immobilization, flow chambers functionalized with amine silane and crosslinked with the amine-DNA through glutaraldehyde, yielding a decorated DNA-surface. The free-biotin end was tethered with Dynabeads™ M-270 coated with streptavidin. The peptide ssIL-8 α (17 aa) was used to study DNA dynamics under forcé.

Results: Normally, the DNA fluctuates due to thermal energy; however, the peak-to-peak signal decreases after injecting the peptide into the solution. We have used signal noise analysis to interpret the binding of the peptide to

the DNA, which is also concentration dependent. Moreover, from polymer physics, the peptide changes the elastic properties of the DNA, making it less extensible after the binding of the peptide; it shortens after peptide binding. **Discussion:** Our results suggest that through a simple two-step chemistry, it is possible to implement an assay sensitive to the formation of DNA-macromolecule complexes. Moreover, further experimentation could allow to access kinetics constants.

Acknowledgment: FONDECYT 1221064

P34. NUAK1 promotes pentose phosphate pathway via hnRNPK-G6PD and favours oxaliplatin chemoresistance. Tomas Molina¹(tmolina2019@udec.cl), Alejandro Farias¹, Viviana Coliboro¹, Luis Espinoza, Martina Concha, Roberto Amigo, Jean Pierre-Koppling, Daniel Murphy², Violeta Morin¹, Roxana Pincheira¹, Ariel Castro¹.

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Introduction: Metabolic reprogramming is an important hallmark of cancer progression, which includes the upregulation of the pentose phosphate pathway (PPP) in several cancer types. The PPP is pivotal in regulating cell proliferation and chemoresistance, providing precursors for lipid synthesis, proliferation, and oxidative stress (ROS) response. Previous studies showed that NUAK1 increases PPP metabolites, such as 6-phosphogluconate and NADPH. NUAK1 is a serine/threonine kinase widely expressed in cancer, implicated in survival, proliferation, migration, metabolism, and gene expression. Also, previous analyses suggest that hnRNPK could be a novel interactor of NUAK1. Interestingly, HnRNPK is a ribonucleoprotein that inhibits the splicing of glucose-6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme of PPP

Materials and Methods: To identify NUAK1 interactors, we performed protein–protein interaction assays in colorectal cancer (CRC) cells, focusing on RNA-binding proteins with known regulatory roles in metabolism. Splicing regulation of G6PD mRNA was analyzed through RNA immunoprecipitation and splicing assays. Metabolomic profiling was conducted to evaluate changes in PPP intermediates, NADPH levels, ROS accumulation, and lipid biosynthesis upon NUAK1 modulation. Additionally, oxaliplatin treatment was applied to assess NUAK1 and G6PD expression dynamics. Finally, patient-derived CRC organoids were used to evaluate the functional impact of NUAK1 inhibition on G6PD expression, proliferation, and drug response.

Results: Metabolomic profiling further revealed that NUAK1 expression enhances the accumulation of PPP intermediates and favors lipid biosynthesis. Conversely, NUAK1 inhibition led ROS accumulation, and decreased NADPH levels. Importantly, oxaliplatin treatment induced the expression of both NUAK1 and G6PD, suggesting that this axis is activated as an adaptive mechanism to support oxaliplatin resistance. Building on these observations, we investigated patient-derived colorectal cancer organoids, where NUAK1 inhibition reduced G6PD expression, impaired proliferation, and sensitized organoids to oxaliplatin, confirming the functional relevance of this pathway in a clinically relevant setting.

Discussion: Together, our study uncovers a mechanistic role of NUAK1 in metabolic rewiring through the regulation of PPP, highlighting its induction under chemotherapy and supporting NUAK1 as a potential therapeutic target to overcome oxaliplatin resistance in colorectal cancer.

Acknowledgment: Fondecyt 1241771, Fondecyt 1201215, VRID DICA N°170/25

P35. Characterization of Aquaporins in *Eucalyptus* spp under Water Stress. Gustavo Morales-Baeza (gmorales20@alumnos.otalca.cl); Martin Parada-Zapata; Felipe Valenzuela-Riffo; Raúl Herrera.

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Introduction: *Eucalyptus* is an angiosperm with economic importance for Chile forest industry showed a limitation in development and growth due to deficiency in water availability. In this context, molecular analyses are key to evaluating the pattern of response involved to water deficit. Aquaporins (AQPs) are a genes family of interest, as they allow or facilitate the flow of water or small solutes across the plasma membrane. In this sense, AQPs play a crucial role in the hydration at cellular level. In this work, a search for the AQP family was carried

out in *Eucalyptus globulus* and two members of this family were characterized, together with an analysis of the relative accumulation of transcripts in young seedlings exposed to drought.

Materials and Methods: The search and filtering of AQP sequences were carried out, using HMMER against the reference genome of *E. globulus*. Subsequently, two of these sequences were selected to perform RT-qPCR assays to determine their transcriptional profile, as well as the subcellular localization in *Allium cepa* cataphylls.

Results: Forty-eight putative AQP members were found, and the presence of *cis*-elements, synteny, and relevant residues were analyzed. The two selected proteins showed significant changes in the accumulation of transcripts for seedlings exposed to water stress. Whereas EglPIP2;4 showed variations among genotypes and gradual changes at 6, 8 and 10 days, EglTIP1;4 exhibited a similar behavior in all evaluated genotypes. The subcellular localization for both showed their presence in vacuole and plasma membrane for TIP and PIP, respectively.

Discussion: The results obtained demonstrate that both water transporters could be involved in the response mechanisms and possible adaptation to water stress in *Eucalyptus spp.*

Acknowledgment: Anillo ATE 220043 and ANID-Fondecyt 1241579

P36. Poldip2 Deficiency Promotes OGT-Dependent Microtubule Destabilization and SRF Stabilization in Vascular Smooth Muscle Cells. Francisca Muñoz Cortés¹(fmuez@uc.cl), Felipe Paredes² and Alejandra San Martín^{1,2}.

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Introduction: Maintenance of differentiated phenotype of vascular smooth muscle cells (VSMCs) protects against vascular diseases such as atherosclerosis and aneurysms. Our laboratory has shown that deficiency of the mitochondrial protein Poldip2 induces glycolytic reprogramming and promotes accumulation of serum response factor (SRF), leading to a highly differentiated VSMC phenotype. This mechanism involves O-linked GlcNAc transferase (OGT)-mediated glycosylation and subsequent inhibition of nuclear ubiquitin–proteasome system (UPS). However, how OGT-mediated glycosylation disrupts nuclear proteostasis remains unclear. Since microtubule-dependent structures serve as hubs for nuclear proteostasis, we hypothesize that Poldip2 deficiency promotes VSMC differentiation through OGT-dependent disruption of the microtubule cytoskeleton, thereby inhibiting nuclear UPS activity.

Materials and Methods: Human aortic smooth muscle cells (HASMCs) were transfected with control or Poldip2-targeting siRNA. Microtubule organization was assessed by immunofluorescence using anti- β -tubulin antibody. O-GlcNAcylation was modulated by OGT siRNA or OGA inhibition with Thiamet G. Microtubule glycosylation was analyzed by immunoprecipitation and immunoblotting. UPS activity was measured using the fluorogenic probe Bz-Val-Gly-Arg-AMC. Microtubules were stabilized with taxol and MARK inhibitor. SRF stabilization was evaluated by western blotting. Independent experiments, n = 3–4.

Results: In Poldip2-deficient VSMCs, microtubules were disrupted, an effect rescued by OGT knockdown. Consistently, OGA inhibition with Thiamet G enhanced microtubule glycosylation. Importantly, taxol-mediated microtubule stabilization restored UPS activity in Poldip2-deficient cells. Moreover, taxol and MARK inhibition prevented SRF accumulation in these cells, supporting a role of microtubule integrity in UPS regulation and SRF stabilization.

Conclusion: These findings identify a novel pathway whereby Poldip2 deficiency promotes OGT-dependent microtubule destabilization, leading to UPS inhibition and SRF accumulation. This work links metabolism, cytoskeletal regulation, and VSMC differentiation, suggesting targeting the OGT–microtubule–UPS axis may provide therapeutic opportunities to modulate VSMC phenotype in vascular disease.

Acknowledgment: FONDECYT 1251373

P37. Mass Photometry—a New Technology for Latin America. Karina New (karina.new@postqyf.uchile.cl) and Christian A. M. Wilson.

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Introduction: Mass photometry (MP) is a label-free, single-molecule technique that measures the mass of biomolecules in solution through interferometric scattering microscopy. It does not require of fixation or labelling, enabling direct analysis of all sample components while saving resources. MP has become a reputable approach for characterizing biomolecular interactions, and we aim to extend its potential beyond conventional applications. In recent work, we demonstrated novel uses of MP, including detection of small ligand binding to proteins and the extraction of dimerization kinetics, not previously reported for this technology. With the establishment of the first MP facility in Latin America at the University of Chile, we continue to expand the scope of MP.

Materials and Methods: For the first time, we will investigate how solvent environment affects protein behavior by performing MP studies with water isotopes. In parallel, we are designing a novel MP protection assay that combines scanning cysteine accessibility mutagenesis (SCAM) with methanethiosulfonate (MTS) reagents to probe binding site accessibility and conformational stability in solution.

MP is also well positioned to compliment the fast-growing field of computational predictions as a rapid and direct way to validate or refine these models. At the same time, MP serves as a reliable platform for sample quality control, critical for all biochemical and biophysical studies.

Results: Through this work, we aim to adapt MP to directly study the relationship between protein folding and aggregation, providing dynamic insight into how misfolding transitions into oligomeric or aggregated states. We will investigate whether the length and distribution of intrinsically disordered regions dictate protein self-assembly, providing fundamental insight into the molecular origins of protein misfolding and disease.

Discussion: These directions highlight MP as a methodological frontier and a versatile platform for addressing challenges in protein science, while providing regional researchers access to an advanced platform for molecular characterization.

Acknowledgement: FONDEQUIP 240012

P38. Regulation of MYC by the Transcription Factor SALL2 in Colorectal Cancer Cells. Ebonny Olivera^{1,2} (eoivera2020@udec.cl), Roxana Pincheira¹, Valentina González-Pecchi²,

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Introduction: Colorectal cancer (CRC) is one of the most frequent neoplasms worldwide, where the MYC oncogene is often overexpressed. SALL2, a transcription factor described as a tumor suppressor, regulates genes involved in cell cycle arrest and apoptosis. However, its functional relationship with MYC has not yet been explored in CRC.

This study aims to determine the role of SALL2 in the protein expression and regulation of the transcriptional activity of MYC in CRC cells.

Materials and methods: SW480 cells with a Tet-On system for inducible expression of SALL2 were used. Induction was performed by treatment with doxycycline (DOX). Protein levels of SALL2 and MYC were validated by Western blot, and the transcriptional activity of MYC was evaluated with a Dual-Glo® Luciferase Assay, normalized to Renilla.

Results: Western blot analysis confirmed the induction of SALL2 after DOX treatment, which showed a slight decrease in MYC levels. Meanwhile, the reporter assays demonstrated that the induction of SALL2 did not significantly reduce MYC-dependent transcriptional activity compared to non-induced controls.

Discussion: Although the effect was not significant, SALL2 could influence MYC indirectly. Both factors share target genes related to cell cycle control and apoptosis. Thus, SALL2 might modulate MYC through these common pathways rather than through direct repression. Furthermore, SW480 cells harbor multiple oncogenic mutations, which could mask potential regulatory interactions. It will be necessary to evaluate other CRC cell lines and analyze the expression of MYC target genes to better clarify this functional interaction.

Acknowledgments: FONDECYT initiation 11251043 (V.G-P)

P39. Characterization of sex-related baseline differences in neonatal rat cardiomyocytes. Ingrid Oyarzún^{1,2} (ingoyarzun@uc.cl), Magdalena Jiménez^{1,2}, Georhan Mancilla^{1,2,3,4}, Pablo Castro^{1,2}, Hugo Verdejo^{1,2}, Clara Quiroga^{1,2}.

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Introduction: Cardiovascular diseases (CVD) are the leading cause of death worldwide and are known to exhibit sexual dimorphism. Mortality from CVD is higher in women than in men, and sex differences are also reflected in the impact of risk factors and in the greater prevalence of adverse drug reactions in women. Although the underlying mechanisms are not fully understood, sex-dependent variations in gene expression have not been systematically explored. To address this gap, we evaluated genes involved in different signaling pathways, with the aim of assessing sex-specific differences in basal expression in neonatal cardiomyocytes. This approach may help clarify how sex influences cardiac biology, beyond hormonal effects, and guide the development of targeted therapeutic interventions.

Materials and Methods: Primary neonatal cardiomyocytes were isolated from the hearts of 1- to 3-day-old Sprague–Dawley rats and sexed accordingly. Gene and protein expression were assessed using RT-qPCR and Western blot, focusing on stress response, signaling, and structural proteins.

Results: Expression analyses included proteins related to quality control mechanisms, both autophagic (ATG5, Beclin-1, LC3) and proteasomal (PSMB8, PSMB5, RPN6), as well as those involved in sarcomere structure and contractility (Titin, Troponin T2), and damage response pathways such as senescence (p21). While most markers showed no statistically significant differences, trends in basal expression were observed. Notably, ATG5, a regulator of autophagy activation, displayed lower expression in male-derived cells compared to female-derived cells.

Discussion: These findings provide preliminary evidence of sex-related baseline differences in cardiomyocytes, which may influence stress responses and disease susceptibility. Our results lay the groundwork for the development of sex-specific therapeutic and diagnostic strategies for CVD, contributing to more personalized and effective interventions.

Acknowledgment: FONDAP-1523A0008 (CQ-PC-HV) and PUENTE-UC 2024-7 (CQ).

P40. Characterization of differentially expressed invertases in *Eucalyptus* spp. under water stress. Martín Parada-Zapata (mparada20@alumnos.utalca.cl), Gustavo Morales-Baeza, Felipe Valenzuela-Riffo, Raúl Herrera. Laboratorio de Fisiología Vegetal y Genética Molecular, Instituto de Ciencias Biológicas, Universidad de Talca

Introduction: *Eucalyptus* spp. is a forest species of high commercial value in Chile, widely used in the timber and paper industries. Water stress triggers physiological and molecular responses that vary among *Eucalyptus* species and can cause industrial losses by affecting product quality and decreasing the survival rate of organisms from this species. A common response is osmotic adjustment, mediated by compounds such as proline, polyols, and soluble sugars. The accumulation of these sugars may be regulated by the invertase enzyme family, which hydrolyzes sucrose into its monomers. This study aims to characterize invertase genes and to determine whether they are key in the response to drought of different *Eucalyptus* spp. genotypes.

Materials and methods: Members of the Invertase gene family were identified from the *Eucalyptus globulus* genome. The chromosomal positions, promoter region, and coding regions were extracted to predict protein products. The gene sequences obtained were compared with a drought-stress transcriptome of *E. globulus*, previously published to identify differentially expressed genes. Selected genes were analyzed by qRT-PCR within the different genotypes. A structural model was built for the differentially expressed protein under drought.

Results: The invertase family comprises 14 members: 2 vacuolar (VINV), 8 cell wall (CWINV), and 4 cytoplasmic (CINV). The gene structure showed conservation in the intron–exon number among subfamilies. The promoters of the genes mainly contain cis-elements responsive to hormones and stress. In the genotypes analyzed, VINV genes showed significant accumulation of transcript under drought. The constructed structural model of the protein shows an architecture mainly composed of β -sheets, with a pocket that houses the catalytic motifs at the center of the structure.

Discussion: These findings suggest that invertases contribute to drought response in *Eucalyptus* spp. Most probably, by promoting osmolyte accumulation and metabolic adjustment during prolonged water deficit.

Acknowledgment: Proyecto Anillo - ATE220043 and ANID-Fondecyt 1241579

P41. Two active sites, three activities, one enzyme? biophysical, structural, and kinetic characterization of the ThiDN enzyme from the hyperthermophilic archaeon *Pyrococcus furiosus*. Martín Pereira-Silva (martin.pereira@ug.uchile.cl), Nicolás Fuentes-Ugarte, Victoria Guixé and Víctor Castro-Fernandez.

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Introduction: The de novo biosynthesis of thiamine pyrophosphate (THI-PP) requires the independent synthesis of 4-amino-5-hydroxymethyl-2-methylpyrimidine pyrophosphate (HMP-PP) and 4-methyl-5- β -hydroxyethylthiazole monophosphate (THZ-P), which are subsequently condensed by enzymes with thiamine phosphate synthase (TPS) activity, generating PP_i and thiamine phosphate (THI-P). Specifically, the HMP-PP biosynthesis involves two consecutive phosphorylation steps of 4-amino-5-hydroxymethyl-2-methylpyrimidine (HMP) by ThiD enzymes that catalyze both reactions in a single active site (HMPK/HMPPK activity). Two domains with TPS activity have been described: 1) ThiE, with a TIM barrel α/β structure and distributed among bacteria, plants, and yeasts; and 2) ThiN, with an $\alpha/\beta/\alpha$ sandwich architecture found in some extremophilic bacteria and archaea (e.g., *Pyrococcus furiosus*). ThiN domains occur in three proteins: 1) ThiR, a fusion with a DNA-binding domain (catalytically inactive); 2) Standalone ThiN; and 3) ThiDN, a fusion of ThiD and ThiN forming a trifunctional enzyme with HMPK, HMPPK, and TPS activities. Here, we present the kinetic and structural characterization of the ThiDN enzyme from *Pyrococcus furiosus* (*Pf*ThiDN).

Materials and Methods: *Pf*ThiDN activities were measured at 70 °C using a thermostable ATP-dependent kinase assay (TIGK/TmG6PDH) to determine K_M and V_{max} for HMPK/HMPPK. TPS activity was confirmed by quantifying PP_i production. The crystal structure of *Pf*ThiDN was solved at 2.9 Å to examine ThiD–ThiN interactions in the presence of HMP-P and ATP _{γ S}. Size-exclusion chromatography, DLS, and SAXS were employed to assess its oligomeric state in solution.

Results: *Pf*ThiDN exhibits HMPK, HMPPK, and TPS activities with defined kinetic parameters, representing the first report of all three activities in a ThiDN enzyme. The 2.9 Å crystal structure revealed interactions between the ThiD and ThiN domains with HMPPK substrates, and biophysical analyses showed that *Pf*ThiDN forms a tetramer in solution, the first reported oligomerization state for a ThiDN protein.

Discussion: These results represent the first comprehensive structural, kinetic, and biophysical characterization of a ThiDN enzyme.

Acknowledgment: Fondecyt N°1221667.

P42. Distinct and Overlapping Contributions of Genetics, Environment, and Their Interaction to Autism-like Behaviors: Evidence from BPA Exposure in the Flailer Mouse.

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Introduction: Autism Spectrum Disorder (ASD) is a heterogeneous neurodevelopmental condition characterized by social and cognitive impairments. Its etiology remains largely unknown, with many cases considered idiopathic. Environmental factors are increasingly recognized as contributors. Bisphenol A (BPA), a chemical widely used in consumer products, has gained attention due to its potential role in ASD predisposition through oxidative stress and mitochondrial dysfunction. We hypothesized that BPA exposure during gestation and lactation exacerbates autism-like behaviors in the Flailer (FLR) mouse model of ASD.

Materials and Methods: FLR and wild-type (WT) female mice were treated with either vehicle (VH) or BPA from breeding until weaning. At 40 ± 2 days of age, offspring were evaluated for anxiety, socialization, motor

coordination, and memory. Hippocampal tissue was collected to assess transcriptional and translational changes in the expression of key genes involved in neurodevelopment, mitochondrial dynamics, and antioxidant function.

Results: The FLR genotype recapitulated ASD-like behaviors, including increased anxiety and reduced repetitive behaviors, among others. BPA exposure in WT mice altered anxiety and memory, whereas in FLR animals, it attenuated social and memory deficits, indicating a phenotypic shift in the FLR×BPA group. At the molecular level, a reduction in the expression of proteins related to neuronal maturation, synaptic plasticity, and mitochondrial biogenesis was observed, while no consistent changes were detected at the mRNA level.

Discussion: These findings suggest that genetic susceptibility in the FLR model, combined with perinatal BPA exposure, generates diverse phenotypic outcomes. This underscores the importance of evaluating toxicant and environmental exposures during critical stages of neurodevelopment across different genetic backgrounds to better understand the pathophysiology of ASD.

Acknowledgment: This project is funded by ANID FONDECYT 1220480 (GA), 1230195 (VP), 1250955 (FJB); MILENIO NCN2023_32 (FJB); UNAB DI-06-24/REG (FJB).

P43. Evaluating Tumor Immune Microenvironment through Immunoscore and Microsatellite Instability in Colorectal Cancer. [Aracelly Quiroz \(aracellyquiroz@udec.cl\)](mailto:aracellyquiroz@udec.cl)¹, Constanza Sanhueza², Bastián Aravena², Carolina Delgado³, Claudio Benavides⁴, Emilia Escalona⁵.

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Introduction: Colorectal cancer (CRC) is a major contributor to cancer-related deaths both globally and in Chile. The traditional TNM staging system offers limited precision in prognosis, whereas immune and molecular markers like Immunoscore (IS) and microsatellite instability (MSI) are gaining traction as effective tools to improve patient classification. IS measures density of inflammatory cells within the tumor's core (CT) and its invasive edge (IM), while MSI-H tumors are marked by a high mutation rate and increased immune infiltration. The objective of this study was to evaluate the interplay between IS and MSI and to determine their joint contribution to tumor microenvironment characterization through digital pathology.

Materials and Methods: A retrospective analysis was conducted on 100 CRC samples from Hospital Guillermo Grant Benavente. Immunohistochemistry for CD3, CD8, and CD45 was carried out, with digital slides scanned using MoticEasyScan and processed through QuPath software alongside pathologist evaluations. IS was determined using percentile-based stratification (IS0–IS4). MSI status was identified through MMR protein immunohistochemistry (MLH1, MSH2, MSH6, PMS2). Automated digital analysis was compared to pathologist evaluations for validation.

Results: There was high agreement between digital and visual evaluations ($R^2 = 0.978$ for CD45; $R^2 = 0.959$ for CD3). In total, 69% of patients had low IS (IS1–IS2), while 31% had high IS (IS3–IS4). Among MSI-H patients, 71% exhibited high IS, indicating significant immune activation. On the other hand, 81% of MSS patients had low IS, suggesting a "cold" tumor microenvironment. Notably, there were exceptions, with some MSI-H tumors showing low IS and some MSS cases presenting high IS, highlighting the complementary nature of these biomarkers.

Discussion: Combining IS and MSI provides a broader characterization of the CRC immune landscape, offering valuable prognostic information. Their integration into routine diagnostic workflows could enhance risk stratification and guide more accurate clinical decision-making.

Acknowledgment: This research was supported by institutional resources, ANID grant #SUC250067, and FONDECYT grant #11251816.

Sponsored by: Roxana Pincheira

P44. Role of ubiquitination blockade of the N-terminus of endothelin-converting enzyme-1c in its

subcellular localization and triggering of a malignant phenotype in colon cancer cells. Paula Romero-Vicencio (paula.romero.1@ug.uchile.cl), María de los Ángeles Toro, Karla Villalobos-Nova, Javiera Vargas, Julio C. Tapia.

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Introduction: The endothelin-converting enzyme-1c (ECE-1c) has been shown to play a role in tumor progression in different types of cancer. Several studies have reported that post-translational modifications at its N-terminus, such as CK2-mediated phosphorylation and ubiquitination, regulate its stability and promote an aggressive phenotype in colorectal, glioblastoma and lung cancer cells. The aim of this study was to evaluate whether the blockade of protein degradation could alter its subcellular localization and promote the acquisition of a malignant phenotype in colon cancer cells.

Materials and Methods: DLD-1 colon cancer cells were transfected with plasmids that express a fusion protein of the N-terminus of two different forms of ECE-1c with the GFP. The forms used were a resistant to proteasomal degradation (ECE-1c^{K6R}) and the wild-type enzyme (ECE-1c^{WT}), both compared with cells transfected with an empty plasmid (i.e. mock) for control. To assess subcellular localization, GFP presence was performed by confocal fluorescence microscopy by comparing it with specific organelle markers. The stability of both NT^X-GFP proteins was evaluated by a cycloheximide assay, in presence and absence of the CK2 inhibitor, Silmitasertib. Finally, to assess the acquisition of a malignant phenotype, different assays were performed to evaluate cell proliferation, migratory capacity, and chemoresistance of both NT^X-GFP expressing DLD-1 cells.

Results: A change in localization of the NTK6R-GFP protein was observed, showing distribution in RER and a granular pattern associated with endosomes. The N-terminus of ECE-1cK6R enhanced GFP's stability in the presence of Silmitasertib, compared with the wild-type enzyme. Increased proliferation and chemoresistance were observed in NTK6R-GFP expressing cells compared to NTWT-GFP cells.

Discussion: Our results demonstrate that blockage of ubiquitination at the N-terminus of ECE-1c, in addition to increasing its stability, promotes localization in RER and potentially endosome structures, which would be related with the acquisition of a malignant phenotype in colon cancer cells.

Acknowledgment: FONDECYT grant #1220353 (JCT).

P45. MicroRNAs modulation by isodrimeninol from *Drimys winteri* (Canelo) in periodontitis associated cellular models: preliminary results. Nelia M. Rodríguez^{1,2}, Pía Loren^{1,2}, Isis Paez³, Viviana Burgos⁴, Constanza Martínez-Cardozo^{5,6}, Alejandra Chaparro^{6,7} & Luis A. Salazar^{1,2} (luis.salazar@ufrontera.cl).

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Introduction: Periodontitis is a chronic inflammatory disease characterized by the progressive destruction of the tooth's supporting tissues, driven by complex interactions between periodontopathogenic bacteria, environmental factors, and the host immune response. MicroRNAs (miRNAs) have emerged as key modulators of inflammatory pathways and are increasingly recognized for their role in the pathogenesis of periodontitis. Their deregulation in this disease suggests potential therapeutic applications targeting miRNA expression. Natural compounds such as isodrimeninol, derived from *Drimys winteri* (Dw), may offer novel approaches to modulate miRNA activity due to their anti-inflammatory properties. However, no studies have previously linked this sesquiterpene to miRNA regulation in periodontitis. The aim of this study was to investigate the *in vitro* effects of isodrimeninol on six miRNAs (miR-17-3p, miR-21-3p, miR-21-5p, miR-146a-5p, miR-155-5p, and miR-223-3p) associated with periodontitis using two cellular models.

Materials and Methods: Saos-2 cells (osteoblast-like cells) and periodontal ligament-derived mesenchymal stromal cells (hPDL-MSCs) were stimulated with lipopolysaccharide (LPS) to induce inflammation and treated with isodrimeninol and resveratrol for comparison.

Results: Isodrimeninol reduced Interleukin-1beta (IL-1 β) and Interleukin-6 (IL-6) gene expression and caused differential expression patterns of the miRNAs examined, upregulating miR-146a-5p and miR-223-3p, while downregulating miR-17-3p, miR-21-3p, miR-21-5p, and miR-155-5p ($p < 0.05$).

Discussion: These findings indicate a connection between miRNAs, periodontitis, and the regulation of inflammation by isodrimeninol, providing potential opportunities for the treatment. However, further validation is needed to confirm these results.

Acknowledgment: Project “Inicia tu Centro” (Grant Number UIN23-0003).

P46. *RUNX2* gene expression in human astrocytic cells: examining association with an active epigenetic profile at the *RUNX2*-P2 promoter and the role of the *RUNX2* factor at target genes. [Vanessa Segura](mailto:v.segurabobadilla@uandresbello.edu)^{1,2} (v.segurabobadilla@uandresbello.edu), Vicente Apablaza^{1,2}, Constanza Mercado^{1,2}, Víctor Pola-Veliz^{1,2}, Sebastian Arredondo^{1,2}, Juan Fuentes^{1,2}, Brigitte van Zundert^{1,2} and Martin Montecino^{1,2}.

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Introduction: Transcription factor *RUNX2* has been proposed to play an important regulatory role during early astrocyte lineage commitment, as it may modulate the expression of downstream pro-astrocytic genes. Here, we assessed molecular mechanisms controlling the expression of the *RUNX2* gene in human astrocyte-like glioma cells by defining epigenetic parameters associated with *RUNX2* transcription.

Materials and methods: Epigenetic profiling was assessed by chromatin immunoprecipitation linked to quantitative PCR amplification (ChIP-qPCR) using antibodies against the active histone marks H3K4me3 and H3K27ac, as well as against the repressive mark H3K27me3. In addition, we examined the contribution of the *RUNX2* factor to downstream target gene transcription by performing loss-of-function analysis using specific shRNAs that knockdown *RUNX2* mRNA and protein levels in the astrocytic cells. mRNA expression analyses were carried-out by RT-qPCR using specific primers against *RUNX2* and target gene transcripts. *RUNX2* protein analyses were assessed by both western blot and immunofluorescence analyses, using specific antibodies.

Results: Our results show that *RUNX2* mRNA and protein expression is significantly elevated in human astrocytic cell lines compared to normal human diploid astrocytes. Importantly, this elevated *RUNX2* expression is associated with a significant enrichment of the active marks H3K4me3 and H3K27ac, and poor enrichment of the repressive mark H3K27me3, at the *RUNX2* P2-promoter region, suggesting tight epigenetic control of *RUNX2* gene transcription in these cells.

Discussion: Together, these results support using human astrocytic model cell system to address mechanisms associated with both *RUNX2* gene expression and to study *RUNX2*-mediated regulation of target genes.

Acknowledgement: FONDECYT1251450; NCN2023_032

P47. ByBug Synthetic®: An Innovative Platform for the Production of Therapeutic Proteins in Black Soldier Fly. Daniela Leiva Carvajal, Valentina Varela Silva, Jorge Caballero González, Nicolás Órdenes-Aenishanslins, Valentina Muñoz Zapata, José Del Solar Bou, Daniel Troncoso-Espinosa, [Clara A. Solai](mailto:clarasolari@bybug.io) (clarasolari@bybug.io). ByBug. Av. San Ramón 1580. Coquimbo. Región Coquimbo, Chile.

Introduction: Intensive animal farming faces significant challenges from diseases, which pose a threat to both animal welfare and human health, resulting in substantial economic losses. Traditional pharmaceutical solutions for animal health are often prohibitively expensive. The oral administration of therapeutic proteins through genetically modified insect larvae represents a highly competitive and cost-effective approach. ByBug has developed the ByBug Synthetic® platform for the expression of therapeutic proteins in Black Soldier Fly, *Hermetia illucens*, larvae.

Materials and Methods: The platform's development involved genetic engineering of insects, insect rearing, and protein downstream processing. Protein expression was confirmed through the detection of the recombinant

protein in protein extracts from the modified larvae using western blot and ELISA.

Results: Our platform was validated using two different therapeutic proteins: one for aquaculture and another for the poultry industry. The latter was successfully detected in protein extracts of the recombinant larvae, confirming its expression within the ByBug Synthetic® platform.

Discussion: The results confirm that ByBug Synthetic® is a viable and effective platform for producing therapeutic proteins in black soldier fly larvae. This approach provides a sustainable and cost-effective method for delivering therapeutics orally to animals, offering a new avenue for disease management and nutrition in animal farming. The platform's success and versatility demonstrates its potential to revolutionize the animal health industry.

Acknowledgment: Startup Ciencia 2024. ANID. SUC240043.

P48. Functional loss of TET enzymes reveals their critical role in controlling transposable element expression at heterochromatin in mouse embryonic stem cells. Bruno Soto-Moraga¹ (b.sotomoraga@uandresbello.edu), Gustavo Jara-Fernández¹, Constanza Mardones¹, Sebastián Echeverría-Araya¹, Robert Crawford², Anjana Rao³ and Hugo Sepúlveda^{1,3}. ¹Faculty of Medicine, Institute of Biomedical Sciences, Universidad Andres Bello, Chile. ²Biomodal, The Trinity Building, Chesterford Research Park, Cambridge CB10 1XL, UK. ³La Jolla Institute for Immunology, University of California San Diego, USA

Introduction: Epigenetic modifications are essential to genome stability and to the regulation of both gene and transposable elements (TEs). DNA methylation is catalyzed by DNA methyltransferases (DNMTs) and reversed by Ten-Eleven Translocation (TET) dioxygenases. Dysfunction of TET enzymes leads to complex and time-dependent methylation changes. Our research aims to elucidate the impact of TET depletion on TE activity and transcriptional regulation.

Methodology: We used mouse embryonic stem cells (mESCs) carrying an inducible genetic system that yield a triple TET1/2/3 knockout (*Tet-iTKO*) to assess the role of TET-enzymes on transcription and chromatin features. Gene expression, DNA methylation, and chromatin accessibility were interrogated through RNA-seq, 6-base sequencing and ATAC-seq, respectively. Binding of chromatin-associated proteins were interrogated through ChIP-seq. We focused on transcriptional and chromatin changes at TEs and their influence on nearby genes. Integration of these datasets enabled a global view of transcriptional and epigenetic regulation.

Results: TET-loss-of-function led to marked transcriptional changes with predominant upregulation of genes and TEs. We found that activated gene clusters frequently contained TEs with elevated expression, suggesting a regulatory crosstalk between them. Moreover, TE-families such as MERVL and L1 displayed a clear dependence on TET-proteins for proper transcriptional control, with chromatin accessibility correlating with the transcriptional outcome of the TET-dependent TEs. Remarkably, DNA methylation at promoter regions does not correlate with the transcriptional changes. We found that many upregulated TEs in *Tet-iTKO* mESC were reactivated from heterochromatin, indicating the clear dysfunction of these chromatin domains when TET-functions are altered as has been observed in cancer.

Discussion: Our findings highlight the intricate link between TET activity, TE regulation, and chromatin organization. This work provides new perspectives on the epigenetic control of genome integrity and gene expression, underscoring the potential role of TEs in the etiology of epigenetic disorders.

Acknowledgements: ANID and The Pew Charitable Trusts.

P49. Targeted deletion of a segment in the 3' end of MALAT1 gene impacts transcript levels and stability of MALAT1 and its antisense TALAM1 in AGS gastric adenocarcinoma cells. Valeria Valdés-Estrada¹ (v.valdsestrada@uandresbello.edu), Roberto Munita², Fernando J. Bustos¹, Rodrigo Aguilar¹. ¹Institute of Biomedical Sciences, Faculty of Medicine, Universidad Andres Bello. ²Department of Biochemistry and Molecular Biology, Faculty of Chemical and Pharmaceutical Sciences, Universidad de Chile.

Introduction: Gastric cancer has a high incidence and mortality worldwide. Contributing factors include genetic alterations such as deregulation of long non-coding RNAs (lncRNAs) that promote tumor progression. One well-known lncRNA is MALAT1, which is overexpressed in gastric cancer and drives molecular and cellular changes.

MALAT1 is processed at its 3' end, forming a triple-helix structure that stabilizes the transcript and enables its nuclear accumulation, contributing to high expression levels. MALAT1 also has an antisense transcript, TALAM1, which enhances MALAT1 abundance.

Materials and Methods: CRISPR-Cas9 was used to delete a segment of the 3' end of MALAT1 by designing two guide RNAs. Deletions were verified by PCR and Sanger sequencing. Transcript levels of MALAT1 and TALAM1 were quantified by RT-qPCR, ssRT-qPCR, and dPCR. Transcript stability was assessed in AGS colonies using Actinomycin D treatment.

Results: A segment within the triple-helix region of MALAT1 was successfully deleted. As a consequence, transcript levels of both MALAT1 and TALAM1 in AGS colonies were significantly reduced. Furthermore, the stability of both transcripts decreased markedly compared with control colonies

Discussion: These findings highlight the importance of the triple-helix region as a key determinant of MALAT1 transcript abundance and stability. Moreover, loss of the 3' end segment may disrupt the positive regulatory relationship between MALAT1 and its antisense TALAM1.

Acknowledgment: FONDECYT 1240853, FONDECYT 1250955, FONDECYT 11230662, FONDEQUIP EQM230028

P50. Metabolomic response of *Citrullus lanatus* grafted onto *Lagenaria siceraria* to water stress. Javiera Valenzuela-Herrera¹ (javieravalenzuelaherrer@gmail.com), Claudio Martínez¹, Carlos Maldonado¹, Raúl Flores², Rodrigo Contreras Soto³.

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Introduction: Watermelon (*Citrullus lanatus*), from the Cucurbitaceae family, is one of the most widespread fruit crops in Chile, concentrated mainly in the Metropolitan, O'Higgins, and Maule regions. Due to its high water content (90–92%), it requires abundant irrigation for optimal fruit filling, which makes it sensitive to water deficits. Given this scenario, the use of tolerant rootstocks, such as *Lagenaria siceraria*, is an effective strategy for improving the crop's adaptation to drought conditions.

This study evaluated the interaction between *C. lanatus* and two accessions of *L. siceraria* with contrasting tolerance to water stress (Illapel-tolerant; Filipinas-susceptible) by analyzing metabolites in aerial and root tissues under full irrigation and water deficit conditions.

Materials and Methods: Watermelons (*Citrullus lanatus*) were grafted onto two accessions of *Lagenaria siceraria* with different tolerances to water deficit (Illapel and Filipinas). Two irrigation treatments (full and deficit) were applied, and leaves and roots were collected for comparative metabolomic analysis.

Results: The results showed that the Illapel rootstock activates tolerance mechanisms mainly in the root, while Filipinas induces the protective response in the aerial tissue of the graft. In both cases, stimulation of pathways associated with osmoregulation, redox balance, energy metabolism, and lipid metabolism was detected. In addition, 16 metabolites shared between leaves and roots were identified, mostly related to defense against abiotic stress. These results support the use of *L. siceraria* (mainly Illapel) as a tool to maintain watermelon productivity in climate change scenarios.

Discussion: These experiments demonstrate the use of *L. siceraria* (particularly Illapel) as a tool to maintain watermelon productivity in climate change scenarios.

Acknowledgment: FONDECYT 11240273.

Sponsored by: Nathan Johnson.

P51. Fickian Diffusion–Drug Delivery of Polyphenols from β -Cyclodextrin–Functionalized Porous Silicon Carriers: Toward Osteoarthritis Applications. Humberto Vélez Slimani^{1,3} (h.velez01@ufromail.cl), Jacobo Hernández Montelongo², Luis A. Salazar³.

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Introduction: Osteoarthritis (OA) is a degenerative joint disease marked by cartilage loss and chronic inflammation. Polyphenols such as pinocembrin (PIN) and caffeic acid phenethyl ester (CAPE) have antioxidant and anti-inflammatory potential, yet poor aqueous solubility and short half-life limit their use. The aim of this study was to engineer β -cyclodextrin-functionalized porous silicon (nPSi- β CD) submicron-sized particles for sustained delivery of PIN/CAPE and to analyze release mechanisms relevant to OA-oriented applications using patient-derived osteoarthritic chondrocyte cell lines.

Materials and Methods: nPSi was produced by electrochemical etching and functionalized by in situ β CD polymerization with citric acid. PIN and CAPE were loaded by adsorption. Physicochemical characterization included UV-Vis spectroscopy, dynamic light scattering (DLS), zeta potential, and ATR-FTIR. In vitro release (PBS, pH 7.4, 37 °C, 8 h) was quantified by UV-Vis at 322 nm (PIN) and 324 nm (CAPE). Kinetic models (first-order, Higuchi, Weibull) were fitted to the release profiles.

Results: The ~280 nm nPSi absorption was retained after functionalization/loading. Hydrodynamic size increased from 228.6 nm (nPSi) to 348.0 nm (nPSi- β CD), and to 384.6 nm (PIN) or 563.8 nm (CAPE) upon loading. Zeta potential shifted from -42.9 mV (nPSi) to -30.8 mV (nPSi- β CD) and to -22.3/-24.8 mV after loading. ATR-FTIR showed additional bands consistent with polyphenol incorporation. β CD functionalization slowed release versus non- β CD controls. Weibull provided the best fit ($R^2 > 0.94$), with $\beta \leq 0.75$, indicating predominantly Fickian diffusion in disordered matrices.

Discussion: nPSi- β CD particles enable controlled, diffusion-driven polyphenol release and support their use as delivery platforms for subsequent evaluation in patient-derived osteoarthritic chondrocyte cell lines, focusing on inflammation-related readouts.

Acknowledgment: Fondecyt N° 1230553 and Dirección de Investigación, Universidad de La Frontera (PP24-0003).

P52. *SLC16A11* expression increases under hypoxic conditions in HepG2 cells. Manuel Venegas (mvenegasr@magister.ucsc.cl), Lorena Mardones, Valentina González-Pecchi, Marcelo Villagrán. Laboratorio de Investigación en Ciencias Biomédicas, Facultad de Medicina, Universidad Católica de la Santísima Concepción, Concepción, Chile.

Introduction: A haplotype of 18 single nucleotide polymorphisms (SNPs) in *SLC16A11* is strongly associated with type 2 diabetes (T2D) risk and functions as an expression quantitative trait locus (eQTL) in human hepatocytes. This association is mainly driven by two promoter SNPs and one variant in the 5'UTR. In the hepatic lobule, metabolic zonation has been described, largely influenced by oxygen availability. The pericentral zone is characterized by physiological hypoxia (4% O₂), whereas periportal regions display oxygen concentrations up to four-fold higher. We hypothesized that oxygen availability may act as a physiological regulator of *SLC16A11* expression in hepatocytes.

Methods: We performed an *in silico* analysis of transcription factor binding motifs using the Eukaryotic Promoter Database (EPB) from the ExPASy server, focusing on regulatory sites for HIF1 α . In parallel, we developed an in vitro hepatic zonation model by culturing HepG2 cells under normoxia (20% O₂) or hypoxia (4% O₂). The *SLC16A11* locus was genotyped by Sanger sequencing, while gene and protein expression were assessed by RT-qPCR, western blot, and immunofluorescence.

Results: Bioinformatic analysis identified putative binding sites for HIF1 α and SP1. No SNPs colocalized with HIF1 motifs; however, one SNP overlapped with an SP1 site, predicted to alter its binding capacity in silico. HepG2 cells carried the reference *SLC16A11* haplotype. Under hypoxic conditions, *SLC16A11* expression was significantly upregulated, showing a two-fold increase by western blot and a three-fold increase by RT-qPCR. Hypoxia induction was validated by a three-fold increase in HIF1 α protein levels.

Discussion: Our in vitro hypoxia model was validated, as it consistently induced both HIF1 α and *SLC16A11*. These findings suggest that oxygen concentration contributes to the regulation of *SLC16A11* expression in

hepatocytes and provide a foundation for downstream functional studies to explore the role of hypoxia in the genetic risk of T2D.

Acknowledgments: Fondecyt 11251393 (ANID) and DI-Reg 07/2024, Dirección de Investigación, Universidad Católica de la Santísima Concepción.

P53. Endothelin A receptor-mediated Src activation drives aggressiveness in gallbladder cancer cells.

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Introduction: Gallbladder cancer (GBC) is the most frequent malignancy of the biliary tract and presents high incidence and mortality rates in Chile, largely due to the absence of defined symptoms in early stages. Therefore, the identification of novel therapeutic targets to reduce malignant progression is of great importance. One pathway associated with this process is the endothelin-1 (ET1) axis, which activates the endothelin type A receptor (ETAR), promoting aggressive features such as cell proliferation and invasion. However, the mechanisms linked to ETAR signaling, mediated by its localization/trafficking, and its functional impact remain poorly characterized. Thus, our aim was to analyze the basal localization of ETAR, the activation of downstream signaling pathways, and the effects of ET1 and its pharmacological blockade on proliferation and invasion in GBC cells.

Methods: Gallbladder cancer cell lines CAVE and NOZ were used as experimental models. ETAR basal localization was analyzed by confocal microscopy. The activation of signaling pathways, including Src and downstream mediators AKT and ERK1/2, was evaluated by Western blot of phosphorylated proteins. Functional effects were assessed through proliferation assays in CAVE and NOZ cells, and invasion assays in NOZ cells using Matrigel-coated Transwell chambers.

Results: ETAR was expressed and displayed ubiquitous localization in both cell models. ET1 stimulation promoted the activation of oncogenic signaling pathways, leading to increased proliferation and invasion in gallbladder cancer cells. These effects were consistently attenuated by pharmacological inhibition, either through ETAR antagonism with ambrisentan, Src inhibition with saracatinib, or the combination of both.

Discussion: The ETAR/Src axis promotes proliferation and invasion in GBC, and its pharmacological inhibition attenuates these phenotypes, highlighting its therapeutic potential.

Acknowledgment: ANID/FONDECYT 1250780 (IN)

P54. Localization of endothelin-converting enzyme-1c in the multivesicular body of colorectal cancer cells.

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Introduction: The endothelin-converting enzyme-1c (ECE-1c) is one of the four isoforms of ECE-1. They differ only by their N-terminus, and it is reported that it determines their subcellular location, being mainly cell membrane and, to a lesser degree, cytoplasmic in the case of ECE-1c. In addition, previous studies in our laboratory have shown that phosphorylations on the N-terminus increase its stability, promoting an aggressive phenotype that favors progression of various cancers, such as glioblastoma, colon and lung cancer. This led us to study whether ECE-1c may be found in the multivesicular body (MVB), suggesting its involvement in cell trafficking through the endosomal pathway and its potential cargo in secreted EVs, which participate in cellular communication.

Materials and Methods: Cells expressing different Flag/myc-tagged ECE-1c proteins mutated at its N-terminus were used. Confocal immunofluorescence microscopy was used to colocalize ECE-1c mutants with markers enriched in the MVB. Z-stack sections were analyzed using Python scripts and machine vision.

Results: All ECE-1c mutants had the same cytoplasmic granular pattern. It was possible to generate Python algorithms which individually segmented cells and allowed 3D reconstruction. The analysis showed a low positive correlation of ECE-1c in MVB.

Discussion: Contrary to what was previously described, ECE-1c had a cytoplasmic granular pattern and is not enriched to the cell membrane. This study localized ECE-1c in the MVB, supporting the idea of its participation in endosomal trafficking and its possible cargo in EVs. However, the low correlation could be attributed to a limited abundance of ECE-1c in this compartment.

Acknowledgment: FONDECYT 1220353 (JCT), ID25i10020 (LLG)

P55. ClpP Activation by Novel Small-Molecules Alter Mitochondrial Proteostasis and VSMC Phenotype. Catalina Yáñez Gomez¹(catalina.yezgmez@gmail.com), Osvaldo Yáñez², Francisca Muñoz Cortes¹ and Alejandra San Martín^{1,3}.

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Introduction: The mitochondrial protease caseinolytic protease P (ClpP) is a highly conserved protease, essential for mitochondrial proteostasis and cellular stress responses. We have reported that pharmacological ClpP activation preserves the differentiated phenotype of vascular smooth muscle cell (VSMC) inhibiting vascular aneurysms. Using in-silico docking studies, we have designed and synthesized two novel ClpP activators. The objective of this study was to evaluate the ability of these two novel small-molecules to activate ClpP proteolytic activity.

Materials and methods: Human aortic smooth muscle cells (HASMCs) were treated with the novel ClpP activators (10 μ M for 24 hours), and the expression of the ClpP canonical substrates succinate dehydrogenase subunit A (SDHA), succinate dehydrogenase subunit B (SDHB), ubiquinol-cytochrome c reductase core protein 2 (UQCRC2), and its regulatory partner ClpX were analyzed by Western blot.

Results: Treatment with both novel ClpP activators (10 μ M for 24 hours) altered mitochondrial proteins levels. Specifically, SDHA, SDHB and UQCRC2 were decreased, while ClpP and ClpX were maintained, indicating a potential impact on mitochondrial respiratory complexes and proteostasis machinery.

Discussion: These findings demonstrate that these compounds induce ClpP activity within the mitochondria. By modulating ClpP activity these compounds may influence VSMC phenotype highlighting their potential as a novel therapeutic strategies for vascular diseases such as aneurysms.

Acknowledgment: FONDECYT 1251373

P56. Lysine monomethylation and ADP-ribosylation act in concert to regulate the association of histones H3 and H4 to ribosomes. Patricio Zapata (pzapata1@correo.uss.cl)¹, Francisco Saavedra¹, Francisca Álvarez¹, Claudia Espinoza¹, Yerko Castillo¹, Rodrigo Villanueva¹, Ignasi Forme², Axel Imhof² & Alejandra Loyola¹.

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Introduction: Histones H3 and H4, essential components of chromatin, require strict coordination of synthesis, modification, and assembly processes to maintain genomic stability and transcriptional regulation. In this study, we explored the role of early modifications, particularly lysine monomethylation (H3K4me1, H3K9me1, H4K20me1) and PARP1-mediated ADP-ribosylation, during histone translation in cytosolic ribosomes.

Materials and Methods: Ribosomal fraction containing nascent histones were analyzed through biochemical assays of protein-ribosome interaction. PTMs were evaluated using molecular and proteomic techniques aimed at characterizing monomethylation and ADP-ribosylation events occurring during translation.

Results: Analyses revealed that these modifications are not secondary events but occur co-translationally and play determinant roles in the cellular fate of nascent histones. Monomethylation at specific residues acts as a recognition signal that regulates histone-ribosome interaction and facilitates PARP1 recruitment. In turn, ADP-ribosylation contributes to the efficient release of histones from the ribosome, promoting progression through the cytosolic maturation cascade and incorporation into chromatin. In the absence of these marks, ribosomal stalling, histone sequestration, translational stress, and defects in chromatin organization were observed.

Discussion: These findings support a model in which the ribosome functions not only as a protein synthesis machine but also as an integrative platform for epigenetic signals. Early modifications orchestrate the connection between translation, protein maturation, and chromatin regulation, expanding our understanding of histone biogenesis and underscoring their relevance as critical nodes for nuclear homeostasis.

Acknowledgment: Fondecyt Regular 1240409, Centro Ciencia & Vida FB210008, Financiamiento Basal para Centros Científicos y Tecnológicos de Excelencia, and San Sebastian University scholarship.

P57. The hepatokine LEAP-2 regulates lipid uptake and mitochondrial dynamics in neonatal rat ventricular myocytes. Sebastián Aedo-Cares^{1,2} (seba.aedo.cares@gmail.com), Sebastián Leiva-Navarrete^{1,2}, Rodrigo Troncoso^{2,3}, Valentina Parra^{1,2,4}.

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Introduction: Metabolic dysfunction-associated fatty liver disease (MAFLD) is characterized by increased cardiometabolic risk, lipid accumulation in hepatocytes, and altered secretion of hepatokines. Liver-expressed antimicrobial peptide 2 (LEAP-2), an endogenous antagonist of ghrelin and inverse agonist of its receptor (GHSR), is markedly elevated in MAFLD. Notably, both GHSR and ghrelin are expressed in cardiomyocytes, where their signaling exerts cardioprotective effects. Here, we investigated the effects of LEAP-2 on neonatal rat ventricular myocytes (NRVMs) and its relevance in palmitate-induced hypertrophy.

Materials and methods: NRVMs were stimulated with LEAP-2 (1 μ M, 24 h) to evaluate its inverse agonist effect. To assess the cardioprotective actions of ghrelin, cells were co-treated with ghrelin (1 μ M) and palmitate (220 μ M, 24 h). Finally, NRVMs were exposed to LEAP-2 (1–10 μ M) with palmitate in the presence or absence of ghrelin to evaluate its antagonistic action and role in palmitate-induced hypertrophy and mitochondrial dysfunction.

Results: LEAP-2 increased NRVM area without elevating canonical hypertrophic markers. It altered lipid metabolism by upregulating CD36 and downregulating CPT1B, CPT2, and lipogenic genes (SREBF1, ACSL1, FASN). LEAP-2 also promoted mitochondrial biogenesis (increased PGC1 α , TFAM, mtHSP70), leading to higher mitochondrial number. Although ATP levels remained unchanged, mitochondrial membrane potential decreased. Ghrelin attenuated palmitate-induced hypertrophy, reducing cell area and hypertrophic markers. LEAP-2 blocked the anti-hypertrophic effects of ghrelin on cell area. However, co-treatment with LEAP-2 and palmitate neither exacerbated hypertrophy nor prevented palmitate-induced mitochondrial dysfunction.

Discussion: These experiments demonstrate that LEAP-2, via its inverse agonist effect, alters NRVM lipid metabolism and mitochondrial dynamics. Moreover, LEAP-2 modulates the cardioprotective actions of ghrelin against palmitate-induced hypertrophy, although it does not directly promote hypertrophy. Further studies are needed to clarify long-term effects of LEAP-2 in MAFLD-associated cardiac dysfunction.

Acknowledgment: This project is funded by ANID FONDECYT 1230195 (VP) and 1250720 (RT).

P58. Pyruvate kinases from methanogenic archaea: structural and kinetic insights into the allosteric AMP activation. Ignacio Aravena-Valenzuela¹ (ignacio.aravena.v@uchile.cl), Sebastián M. Muñoz¹, Antonia Alarcón-Saavedra¹, Guy Schoehn², Víctor Castro-Fernández¹, Victoria Guixé¹.

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Introduction: Pyruvate kinase (PK) catalyzes the final step of glycolysis, being an enzyme subject to several regulatory mechanisms, including allosteric effectors such as fructose-1,6-bisphosphate in bacterial and eukaryotic enzymes and AMP in bacterial and archaeal PKs. Among archaeal methanogenic organisms, *Methanocaldococcus jannaschii* PK (MjPK) is described as activated by AMP, whereas *Methanococcus maripaludis* PK (MmPK) has been reported as not activated.

Material and Methods: MjPK and MmPK were recombinantly expressed, purified, and characterized. In addition, we determined the structure of MmPK in the absence of ligands by X-ray crystallography, as well as its cryo-EM structure in the presence of PEP and AMP. Also, the cryo-EM structure of MjPK in the presence of PEP and AMP was obtained.

Results and Discussion: Kinetic characterization shows that both enzymes display cooperative kinetics for phosphoenolpyruvate and that AMP activates both by reducing the half-saturation constant for PEP. These structures identify the structural determinants of AMP binding in methanogenic PKs, confirm that AMP binds to the canonical allosteric site similarly to other sugar phosphates, and provide mechanistic insights into the regulatory features conserved across this archaeal order. Furthermore, we identified an intramonomer disulfide bond in the lid domain of MmPK that closes over the active site—the first such bond reported for any PK. Given the anaerobic physiological context in which MmPK functions, these cysteines are expected to remain reduced. Consistently, the addition of a reducing agent enhances the activating effect of AMP, suggesting that the disulfide bond modulates the enzyme's sensitivity to allosteric regulation in response to the cellular redox state. Overall, our findings highlight AMP as a key regulator of glycolytic metabolism in methanogenic archaea, as it also activates the glucokinase and phosphofructokinase activities of these organisms.

Acknowledgment: FONDECYT 1231263

P59. The transcription factor Bcin11g05790 is a central regulator of virulence factors in *Botrytis cinerea*. Nicolás Arias-Inostroza^{1,2,3} (nicolasariasinsostroza@gmail.com), Gabriel Pérez-Lara^{1,2,3}, Valentina Hadler-Palma^{1,3}, and Paulo Canessa^{1,3}.

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Introduction: *Botrytis cinerea* is the most important necrotrophic phytopathogenic fungus. Globally, it infects over a thousand plant species, resulting in substantial losses in economically important crops. Its infectivity largely depends on the secretion of protein virulence factors (VFs) that can disrupt or not the plant defense mechanisms. However, their functional study is challenging due to functional redundancy, as deleting individual genes often provides limited information. A promising alternative is to concentrate on transcription factors (TFs) that control the expression of multiple VF-related genes.

Materials and Methods: In this study, a bioinformatics approach was used to predict protein VFs in the *B. cinerea* proteome. These candidates were filtered based on gene expression data during plant infection, resulting in 281 potential VFs. These were incorporated into a reference gene regulatory network (GRN) of *B. cinerea*, identifying a subset of VFs and their potential TF regulators. From this analysis, the gene Bcin11g05790 was identified as a key TF regulating 20 candidate VFs. A loss-of-function mutant strain (Δ Bcin11g05790) was created using homologous recombination, and its infective ability was tested on the leaves of three plant species.

Results: The mutant strain exhibited significantly reduced virulence compared to the WT strain, confirming the TF's role in virulence. Interestingly, the mutant was also more susceptible to the fungus *Trichoderma atroviride*, a biocontrol agent. This result suggests that this TF may play a dual role in both plant-fungal and fungal-fungal interactions.

Discussion: These findings highlight the utility of GRN approaches in identifying novel determinants of virulence, offering a valuable strategy for understanding fungal virulence and the discovery of new targets in *B. cinerea* that reduce virulence while enhancing its biocontrol.

Acknowledgment: This research was funded by ANID-Ph.D. national scholarships 2025-21251665 to N.A.-I. and by the ANID-FONDECYT grant number 1240742 to P.C.

P60. Enrichment of cancer and inflammatory pathways upon knockdown of Antisense noncoding mitochondrial RNA (ASncmtRNA). Juan Barraza Zavala¹ (j.barrazazavala@uandresbello.edu), Christopher Fitzpatrick¹, Gino Nardocci², Verónica Burzio¹.

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Introduction: In contrast to normal proliferating cells, human tumor cells downregulate the expression of antisense noncoding mitochondrial RNA (ASncmtRNA), derived from the mitochondrial 16S gene. Knockdown of ASncmtRNA with an antisense oligonucleotide (ASO-1537) induces selective cell cycle arrest and apoptotic death in tumor cells, while normal cells remain unharmed. Among other molecular effects, this treatment elicits a marked upregulation of the mitochondrial microRNAs miR-4485-3p and miR-1973, contained in the ASncmtRNA sequence. In addition, a transcriptomic analysis performed in the triple negative breast cancer cell line MDA-MB-231 transfected with ASO-1537 or a control ASO (ASO-C), showed many differentially expressed (DE) mRNAs of proteins involved in cell cycle progression, apoptosis and DNA damage repair, among others. To elucidate the mechanisms underlying the selective apoptosis in tumor cells, we sought to map out the potential cellular pathways affected by this treatment and their association with DE microRNAs.

Methods: Enrichment analysis was performed using the differentially expressed genes from the transcriptomic data mentioned above. This analysis was carried out using GSEA and Enrichment map from curated databases and compared with predicted gene targets for overexpressed microRNAs.

Results: Enrichment maps revealed supercluster pathways related to RAS, the cell cycle spindle assembly checkpoint and innate immunity-related pathways. The latter showed significant enrichment values for pathways related to Toll-like receptor, NFkB activation and Interleukin-6. Associations of pairs of overexpressed microRNAs are presented.

Discussion: Several mitochondrial microRNAs have been linked to cancer and several inflammatory pathologies of varied origin including viral diseases. Enrichment analysis of ASncmtRNA knockdown showed modulation of cancer, cell cycle and immune pathways. Genes that participate in these pathways contained predicted and experimental targets of mitochondrial miRNAs hsa-miR-4485-3p and hsa-miR-1973, suggesting that ASncmtRNA articulates responses involved in severe pathologies.

Acknowledgment: FONDECYT 1230760

P61. Partial purification of β -glucosidase from rosehip (*Rosa spp.*) and determination of its anthocyanin-degrading activity. Antonia Beltrán¹ (abeltran2019@udec.cl), Maximiliano Figueroa¹, Amparo Uribe¹, Romina Romero², Juan Roman¹.

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Introduction: Rosehip (*Rosa spp.*) is one of Chile's most exported non-timber forest products. Its nutritional value is mainly attributed to bioactive compounds such as anthocyanins, responsible for the fruit's pigmentation and health-promoting properties. However, anthocyanins are highly unstable and can be degraded by environmental factors and enzymatic activity during fruit ripening. Among the enzymes involved, β -glucosidase has been reported as a key catalyst in anthocyanin degradation by hydrolyzing the β -glucosidic bond between the anthocyanidin and glucose, losing both organoleptic and nutritional qualities. While anthocyanin content in rosehip has been documented, the enzymatic mechanism underlying their degradation remains unexplored.

Materials and Methods: β -glucosidase was partially purified from rosehip by ammonium sulfate precipitation, ultrafiltration (Amicon Ultra-15), and size-exclusion chromatography on a SuperdexTM 75 column. The enzyme was characterized with the pNPG kit to determine optimal pH, temperature, and kinetic parameters (K_m , $V_{m\acute{a}x}$). Anthocyanin content was analyzed by HPLC UV-Vis (Hitachi Chromaster, LiChroCART-LiChrospher RP-18, 25 \times 4 mm) at 520 nm, using water with 1% formic acid and acetonitrile as mobile phases.

Results: Partial purification of β -glucosidase yielded a protein fraction with detectable hydrolytic activity against anthocyanins. Preliminary enzymatic assays indicated maximum activity at acidic pH and moderate temperature, consistent with typical plant β -glucosidases. HPLC UV-Vis analysis confirmed the presence of anthocyanins in rosehip extracts, which showed a trend of decrease upon incubation with the enzyme-enriched fractions, suggesting a potential role in anthocyanin degradation.

Discussion: These findings provide new evidence of β -glucosidase activity in rosehip and its involvement in anthocyanin degradation. Understanding the enzymatic mechanisms responsible for losing bioactive compounds in fruits is essential for developing strategies to preserve their nutritional and functional properties. This study contributes to the biochemical characterization of rosehip and highlights β -glucosidase as a potential target for

modulating anthocyanin stability.

Acknowledgment: The authors thank VRID N°2023000729INI, ANID/Fondecyt 11241160 projects and ANID/FONDECYT 1230549.

P62. Residues from the acidic band of connexin-36 hemichannels interact with divalent cations. Nicolás Bravo-Acuña^{1,2} (nicolas.bravo@postgrado.uv.cl), Aníbal García², Valeria Márquez-Miranda¹, Juan Carlos Sáez², Fernando Danilo González-Nilo^{1,2}.

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Introduction: The extracellular acidic band of connexin-36 hemichannels includes Asp47, Asp48, and Glu49. Our previous studies shown that the activity of the hemichannel is differently influenced depending on the presence of Ca²⁺ or Mg²⁺. The aim of this work was to determine the role of the negatively charged residues from the acidic band in the interaction with divalent cations Ca²⁺ and Mg²⁺.

Materials and Methods: Protein-membrane-solvent systems were prepared with CHARMM-GUI using a structure of the connexin-36 hemichannel obtained from Zenodo (<https://zenodo.org/records/7608483>). Loops were completed using Prime (Schrödinger). Molecular dynamics simulations were performed with NAMD, applying external electric fields in the canonical ensemble. Root-mean-square deviation and numeric density analyses were carried out with CPPTRAJ. Salt bridges analyses were carried out with MDAnalysis. Pore radius of the connexin were calculated with HOLE.

Results: Apart from the cytoplasmic loops, the protein backbone structure remained stable during the trajectories. Our analyses indicates that both cations can participate in non-covalent interactions with residues of the acidic band, but Ca²⁺ also binds residues from outside the pore from the extracellular side. Both Ca²⁺ and Mg²⁺ can form salt bridges within a cutoff of 4.5 Å of the carboxylate groups of Asp47 residues sidechains. Both cations also form sporadic salt bridges with Glu49.

Discussion: Together, our analyses suggest that residues Asp47 of the pannexin-3 heptamer participate in the formation of major non-covalent interactions.

Acknowledgment: FONDECYT 11241081, FONDECYT 1221498, FONDECYT 1231523.

P63. Preparation of Chitin and Keratin Sponges for Biomedical Applications Using a Cost-Effective and Environmentally Friendly Method. Nicole Butto-Miranda (nbutto@uchile.cl).

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Introduction: Natural materials represent an excellent alternative to synthetic ones, particularly in the biomedical field, due to their physicochemical and biological properties as well as their abundance in nature. Chitosan, a polysaccharide derived from chitin, is soluble in acidic media, which facilitates its processing. Keratin, a cysteine-rich protein, contains the RGD and LDV amino acid sequences that promote cell–matrix interactions. These features have led to extensive research on their application in cell culture. In this context, we investigated the fabrication of three-dimensional polymeric scaffolds from keratin and chitosan through a cost-effective and environmentally friendly method, aiming to produce porous materials that may enhance cell infiltration.

Materials and Methods: Low molecular weight chitosan was prepared in acetic acid, while keratin was extracted from Merino sheep wool using NaOH. Sponges were fabricated through sequential freezing at –20 °C (12 h), –80 °C (48 h), and subsequent lyophilization (24–48 h). The obtained samples were characterized by Fourier-transform infrared spectroscopy (FT-IR), optical microscopy, scanning electron microscopy (SEM), and contact angle measurements.

Results: Porous, easily handled sponges were successfully obtained through lyophilization. FT-IR confirmed the presence of the characteristic functional groups of each polymer. Morphological analyses revealed interconnected pores, suggesting potential suitability for biomedical applications.

Discussion: These findings demonstrate the feasibility of obtaining sponges from natural polymers such as chitin and keratin using an inexpensive method that avoids toxic solvents. Furthermore, the technique offers versatility for modifications, enabling the adjustment of final properties to meet specific biomedical requirements, such as cell adhesion, proliferation, and tissue integration.

P64. Advances in the Structural Characterization of Agmatinase-like Protein (LIMCH1). Juan-Pablo Carrasco¹(jcarrasco2019@udec.cl), María-Belen Reyes¹, Diego Bustamante¹, Ignacia Lillo¹, Cristián Villegas¹, Marcell Gatica¹, Maximiliano Figueroa¹, José Martínez¹, José-Yamil Neira², Elena-Amparo Uribe¹.

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Introduction: Agmatine is an amine with neurotransmitter functions and proven anticonvulsant, antineurotoxic, and antidepressant properties. It is metabolized to putrescine and urea by agmatinases or the agmatinase-like protein (ALP), which corresponds to the C-terminal region of LIMCH1. Unlike canonical agmatinases, ALP/LIMCH1 lacks the conserved Mn²⁺-binding residues essential for ureahydrolase activity, and its three-dimensional structure remains unresolved, even using predictive platforms such as AlphaFold. This study aimed to characterize the secondary structure of full-length ΔLIM-ALP and LIMCH1, as well as to evaluate the potential interaction of the calponin homology (CH) domain of LIMCH1 with microtubules.

Materials and Methods: Full-length LIMCH1 and the truncated variant ΔLIM-ALP were expressed and purified using chromatographic techniques. The secondary structure content was analyzed using circular dichroism (CD) spectroscopy. The interaction of the CH domain with microtubules was assessed using an in vitro microtubule-binding assay and immunolocalization in HEK 293 cells.

Results: Circular dichroism analysis revealed that both LIMCH1 and ΔLIM-ALP exhibit a high proportion of disordered regions (~60%) and β-structures (~30%), with minimal α-helices. This contrasts sharply with the α/β/α sandwich fold characteristic of bacterial ureohydrolases, such as E. coli agmatinase. Furthermore, both in vitro and cell-based assays demonstrated that the CH domain of LIMCH1 does not interact with microtubules.

Discussion: The structural profile of LIMCH1 and ΔLIM-ALP differs significantly from that of classical ureohydrolases; however, ALP/LIMCH1 remains the only known mammalian protein with confirmed agmatinase activity. Our findings suggest a distinct structural framework underpinning this enzymatic function and rule out microtubule association in the CH domain of LIMCH1.

Acknowledgment: FONDECYT 1230549

P65. Evaluation of a novel non-invasive therapeutic approach in an experimental model of acute retinopathy. Matías Carrillo¹ (mcarrillo@magister.ucsc.cl), Carlos Farkas¹, Lorena Mardones^{1,2,3}.

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Introduction: Diabetic retinopathy (DR) represents a growing global health burden and remains one of the leading causes of vision loss among working-age adults. Although retinal hyperglycemia and oxidative stress play a key role in the early stages of DR; however, current pharmacological approaches fail to adequately target these mechanisms. Available treatments are mostly invasive and restricted to advanced stages of the disease.

The aim of this work was to evaluate the effectiveness of topical treatment in a model of acute retinopathy, as an initial step toward exploring its potential therapeutic use in early DR.

Materials and Methods: Acute retinopathy (AR) was induced by intravitreal injection of malondialdehyde (10 nM) in BALB/c male mice. The drug was administered topically daily. Retinal changes and visual acuity were assessed daily. After six days of treatment, eyes were enucleated for histological and molecular analysis. Retinal damage was assessed through measurements of lipid peroxidation, DNA damage, and retinal layer thickness. In addition, the expression of GLUT transporters (GLUT1 and GLUT8) was evaluated.

Results: Topical pharmacological treatment reduced retinal lesions by 75% and lipid peroxidation by 30% in the AR model. Moreover, it partially restored the expression of GLUT1 and GLUT8 in different retina layers and decreased the number 8OH deoxyguanosine-positive cells respect to untreated AR eye and mice. On the other hand, the treatment was associated with changes in the thickness of both the inner and outer nuclear layers.

Discussion: The results show potential effectiveness of topical treatment in a RA model which may be extrapolated to its use in early steps of RD and is a less-invasive therapy. This warrants further investigation, particularly to clarify the relevance of the structural change observed.

Acknowledgment: FAEI 08/24, FII03/2024

P66. Integrating Kinetic and Molecular Dynamics Approaches to Elucidate the Allosteric Regulation of a Bifunctional PFK/GK Enzyme in Methanogenic Archaea. Enzo Cevo (enzo.cevo@ug.uchile.cl), Sixto M. Herrera, Víctor Castro-Fernandez, Victoria Guixé. Laboratorio de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Chile.

Introduction: In the modified archaeal glycolytic pathway, enzymes with glucokinase (GK) and phosphofructokinase (PFK) activity use ADP as the phosphoryl donor. In methanogenic archaea, both reactions occur at the same active site of the bifunctional enzyme, ADP-PFK/GK. In our laboratory, we have characterized the allosteric activation of these bifunctional enzymes by their product, AMP. Previously, we determined a high-resolution (1.6 Å) crystal structure of the bifunctional PFK/GK enzyme from *Methanothermococcus thermolithotrophicus* (MttPFK/GK), which revealed the allosteric site responsible for AMP-mediated activation.

Materials and Methods: In this work, we analyzed the role of residues of the allosteric site that establish interactions with AMP using molecular simulation, site-directed mutagenesis, and enzyme kinetics. We focused on Tyrosine-56 and Arginine-58, which were identified through molecular dynamics simulations as critical for ligand interaction, to explore their roles, we designed the R58A and Y56A mutants. The kinetic characterization of the wild-type (WT) and mutant enzymes was carried out by assessing the AMP activation of the PFK and GK activities under both non-saturating (K_M concentration) and saturating ($\sim 10K_M$) substrate conditions.

To complement the experimental findings, we performed 400 ns atomistic molecular dynamics simulations of the WT, R58A, and Y56A systems, each complexed with glucose and MgADP bound in the active site and AMP in the allosteric site. The binding free energy for AMP and glucose was then calculated using MM/PBSA methods to explain the kinetic results.

Results: Our results show that while the WT enzyme is strongly activated by AMP, the Y56A and R58A mutants show a significant decrease in their enzymatic activity and an attenuated response to the allosteric effector. The simulations aim to elucidate differences in the conformational dynamics of the mutants and to assess the potential disruption of communication between the allosteric and active sites, either through chain rearrangements or alterations in local site flexibility. Collectively, the results indicate that residues Y56 and R58 are not only essential for mediating the allosteric signal upon AMP binding but are also required for maintaining basal catalytic activity.

Discussion: This study provides a dynamic perspective on the communication between the allosteric and active sites in the PFK/GK enzymes, offering structural insights into how mutations in the allosteric site can influence both enzyme regulation and catalysis.

Acknowledgment: Fondecyt 1231263.

P67. Study of the immunity of *Lagenaria siceraria*: Characterization of the response to the bacterial elicitor flg-22. Paz Contreras López¹ (paz.contreras@postgrado.uoh.cl), Rodrigo Contreras⁴, Lorena Pizarro^{1,2,3}.

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Introduction: The perception of microbe-associated molecular patterns (MAMPs) is a key component of plant innate immunity. In plants, the receptor FLS2 recognizes bacterial flagellin-derived peptides such as flg-22, triggering early defense responses characterized by a rapid and transient oxidative burst, i.e., the production of reactive oxygen species (ROS) in the apoplast. Here, we aimed to characterize the genetic and functional variability of FLS2 in *Lagenaria siceraria* accessions exhibiting contrasting responses to flg-22.

Materials and Methods: Oxidative burst was quantified in leaf discs from different accessions after flg-22 elicitation via luminometry. Two contrasting accessions (G15, responsive; BG58, non-responsive) were selected for FLS2 transcript and sequence analysis using RT-qPCR and Sanger sequencing. Bioinformatic domain prediction was performed with HMMER. Additional assays with flagellated bacteria (*Pseudomonas syringae* pv. tomato and *Pseudomonas peli* C4) were conducted to assess functional MAMP recognition.

Results: Accessions G15, G21 y Perú showed strong oxidative burst in response to flg-22, whereas BG58 exhibited no significant response. Based on these contrasting oxidative burst profiles, BG58 (non-responsive) and G15 (responsive) were selected for further transcript and sequence analyses. Basal FLS2 transcript levels were similar between both accessions; however, BG58 displayed a significant decrease after flg-22 treatment. Sequencing revealed three non-synonymous mutations (*Tyr864Cys*, *Leu1001Phe*, and *Gly1004Arg*) in the kinase domain of BG58, potentially impairing receptor activation. In bacterial assays, the Perú accession exhibited significantly higher oxidative burst than BG58 when challenged with *Pseudomonas syringae* pv. tomato and *Pseudomonas peli* C4, both flagellated bacteria carrying flg-22.

Discussion: Our findings indicate that structural variation in the FLS2 kinase domain can impair downstream immune signaling without disrupting MAMP perception motifs, explaining the loss of the oxidative burst in response to flg-22 in BG58. This structural variability could influence the effectiveness of bio-stimulants based on flagellated bacteria in cucurbits.

Acknowledgment: This work was funded by the Centro UOH de Biología de Sistemas para la Sanidad Vegetal (BioSaV) de la Universidad de O'Higgins y el proyecto Fondecyt Regular N°1251814.

P68. Conformational dynamics of ancestral ThiD-HMPPK enzymes from mesophilic and thermophilic bacterial lineages. Isaac Cortés-Rubilar (isaac.cortes@ug.uchile.cl), Exequiel Medina, Víctor Castro-Fernández. Laboratorio de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Chile, Santiago, Chile.

Introduction: Thermophilic proteins remain stable at temperatures above 60 °C due to adaptations that allow them to function efficiently under these conditions. One commonly described adaptation is reduced conformational dynamics at room temperature compared to their mesophilic homologs. However, the features that confer thermostability are not universal, as evolutionary history can influence the adaptive strategies employed. Moreover, the correlation between increased thermostability and decreased conformational dynamics in thermophilic proteins has recently been questioned.

Materials and Methods: ThiD-HMPPK enzymes are ATP-dependent phosphotransferases involved in key steps of the bacterial vitamin B1 biosynthesis pathway. In this work, we compared the conformational dynamics of two ancestral ThiD-HMPPK enzymes: one from a mesophilic lineage (Enterobacterales, ancEn, $T_m = 59$ °C) and another from a thermophilic lineage (Thermus, ancTh, $T_m = 87$ °C), using small-angle X-ray scattering (SAXS) and molecular dynamics (MD) simulations.

Results: Kratky analysis of the SAXS data revealed similar global flexibility for both proteins at room temperature. However, ancTh displayed a trend toward reduced flexibility as the temperature approached 50 °C. In contrast, the dynamics of ancEn remained stable up to 50 °C, at which point a slight increase was observed. Gaussian accelerated MD (GaMD) simulations showed overall enhanced conformational dynamics for the thermophilic protein ancTh compared to ancEn at both 300 K and 330 K. Interestingly, certain regions of ancEn, particularly the N- and C-termini, displayed higher fluctuations. Along with it, conventional MD simulations were performed, for the enzyme-ligand ternary complexes, revealing greater flexibility in ancTh at 300 K, which decreased at 330 K.

Discussion: Overall, our results suggest that the thermophilic ancestral enzyme ancTh exhibits increased conformational dynamics at room temperature relative to its mesophilic counterpart, ancEn, and point to a potential link between conformational dynamics and catalytic efficiency in these ancestral enzymes.

Acknowledgment: FONDECYT 1221667, FONDEQUIP EQM200202, FONDEQUIP EQM200266.

P69. Impaired Mitochondrial produced by hypoxia at birth in Rats Monitored by Real Time Seahorse XF: Effect of NAD⁺ replacement. [Díaz-Urbina E.¹ \(eadiaz2@uc.cl\)](mailto:eadiaz2@uc.cl), Almarza C¹, Davidson H¹, Cortés A¹, Lobos P¹, Ezquer F², Morales P^{1,3}, Urra FA¹, Herrera-Marschitz M¹.

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Introduction: Perinatal asphyxia (PA) is a leading cause of neonatal morbidity and long-term neurological impairments. Mitochondrial dysfunction—central to energy production and apoptosis regulation—is highly vulnerable to oxygen deprivation, impairing oxidative phosphorylation and increasing reactive oxygen species, with consequences for brain development and resilience.

Aim: To evaluate the effect of PA on mitochondrial respiration in brain tissue and NAD⁺ replacement as a therapeutic strategy.

Materials and Methods: Organotypic cultures were prepared from rat neonates exposed to global PA or from caesarean-delivered controls. Oxygen consumption rate (OCR) was measured in real time (Seahorse XFe96/Cytation5 integration). Sequential injections of oligomycin (ATP-synthase inhibitor), FCCP (uncoupler), and rotenone/antimycin A (complex I/III inhibitors) were used to estimate basal respiration, ATP-linked respiration, maximal respiration, and spare respiratory capacity. A subset of PA-exposed neonates received nicotinamide riboside (Nrb; 0.8 mmol/kg, i.p., 1 h after delivery).

Results: PA significantly decreased ATP-linked respiration and spare respiratory capacity in substantia nigra and neostriatum, but not in neocortex, suggesting a region-dependent compromise of mitochondrial efficiency and adaptive capacity. Nrb treatment prevented the mitochondrial dysfunction induced by PA.

Discussion: PA compromises mitochondrial function in a region-dependent manner. Early NAD⁺ replacement with nicotinamide riboside preserves mitochondrial integrity and suggests a potential protective strategy against PA-induced brain vulnerability.

Acknowledgment: This study was supported by FONDECYT Chile (No. 1231443, MHM; No. 1231443, FAU; No.1170712, FE; No. 1190562, PMR); FONDEQUIP EQM220164 (FAU, MHM); Puente-ICBM (No570419, PMR).

Sponsored by: Julio Tapia

P70. Human metapneumovirus infection reduces H3K27me3 levels and promotes oncogenic transcriptional programs in lung adenocarcinoma cells. Sebastián Echeverría-Araya¹⁻⁴ (s.echeverraaraya@uandresbello.edu), Josefa Larraín-Navarro², Constanza Mardones², Bruno Soto-Moraga², José Miguel Álvarez⁴, Alejandra San Martín³, Hugo Sepúlveda², Jorge Soto¹.

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Introduction: Patients with lung adenocarcinoma (LUAD) have been shown to be particularly susceptible to infection by human metapneumovirus (hMPV). Our previous RNA-seq analysis indicated that hMPV infection in LUAD epithelial cells may promote an oncogenic transcriptome. We found significant upregulation of KDM6A (hUTX), a demethylase that catalyzes the removal of the repressive histone mark H3K27me3, following hMPV infection. Based on these results, we hypothesized that changes in H3K27me3 are associated with hMPV infection in LUAD cells.

Methods: A GFP-expressing mutant hMPV was used to infect LUAD A549 cells. Global H3K27me3 levels were quantified in GFP-positive A549 cells using immunofluorescence and confocal microscopy, with DAPI as a nuclear counterstain. RNA-seq analysis was performed using the STAR-FeatureCounts pipeline and DESeq2. Gene set enrichment analysis (GSEA) was carried out with oncogenic C4/C6 gene sets to assess correlation with LUAD transcriptional programs. Comparison of H3K27me3 ChIP-seq profiles was performed between non-cancerous lung epithelial cells and A549 cells, both uninfected. ChIP-seq analysis for H3K27me3 was conducted using Bowtie2, Deeptools, and MACS3.

Results: Differential gene expression (DGE) and Gene Ontology (GO) analysis following hMPV infection revealed activation of genes associated with angiogenesis, cell division, and cell migration, as well as a strong

interferon (IFN) response. GSEA showed that hMPV infection in A549 cells positively correlates with a mutant KRAS lung cancer signature. Notably, IFN-inducible genes and established cancer prognostic biomarkers were among the most upregulated transcripts. ChIP-seq analysis demonstrated that H3K27me3 levels at key chemokine genes are higher in cancer cells compared to normal cells. Finally, we observed that global H3K27me3 levels were reduced in hMPV-infected cells, correlating with enhanced UTX expression.

Discussion: This work supports an association between the acquisition of oncogenic features and hMPV infection, highlighting a key role for H3K27me3 changes in these processes.

Acknowledgment: Fondecyt Regular 1231866; The Pew Charitable Trusts, USA.

P71. NUAK1 links metabolic control and transcriptional programs via PFKP/YAP/TAZ axis. Luis Espinoza-Francine (luespinoza2018@udec.cl); Viviana Coliboro-Dannich; Alejandro Farías; Andrea Concha-Vega; Roxana Pincheira; Ariel Castro.

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Introduction: NUAK1, a member of the AMPK-related kinase family, has emerged as a critical regulator of cancer metabolism and signaling. Beyond its canonical roles, NUAK1 interacts with glycolytic enzymes, modulating glucose metabolism. Among them, the platelet isoform of phosphofructokinase-1 (PFKP) has gained attention not only for its central function in glycolysis but also for its ability to translocate into the nucleus. NUAK1 activity facilitates this nuclear translocation, thereby linking metabolic enzymes with transcriptional programs. Once in the nucleus, PFKP interacts with the transcriptional co-activators YAP and TAZ, promoting its target genes. This NUAK1–PFKP–YAP/TAZ axis reveals an unexpected layer of metabolic control that sustains proliferation and tumor progression in breast and colorectal cancers.

Material and Methods: MDA-MB-231 and MCF-7 (BC), and HCT-116 (CRC) cells were treated with NUAK1 specific inhibitor, HTH-01-015, to evaluate nuclear levels of PFKP, TEAD1, YAP and TAZ by Western Blot. Also, YAP targets were measured by RT-qPCR and a luciferase assay to measure TEAD1 activity.

Results: NUAK1 interacts with PFKP in BC and CRC, decreased PFKP activity. Furthermore, we demonstrate that NUAK1 promotes PFKP nuclear localization. Previous studies show that PFKP can interact with proteins that regulate transcription, such as YAP, c-Myc, SOX2, etc. We demonstrate that NUAK1 regulates PFKP-YAP/TAZ interaction, YAP/TAZ nuclear levels and its mRNA targets (Cyr61, CTGF and FoxM1), NUAK1 also regulates TEAD1-dependent transcriptional activity.

Discussion: These findings highlight NUAK1 as a pivotal link between metabolic regulation and transcriptional control. By driving nuclear PFKP and enhancing YAP/TAZ activity, NUAK1 establishes a metabolic–transcriptional circuit that may represent a novel therapeutic vulnerability in cancer.

Acknowledgment: FONDECYT Regular: 1201215 and 1241771, VRID DICA N°170/25.

P72. ANDES virus Gn/Gc spike biogenesis, acid sensitivity and protection in the secretory pathway. Figueroa, Fabián¹ (ffigueroa@cienciavida.org), Nicole Tischler¹, Alfonso González², Claudio Retamal².

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Introduction: Hantaviruses infect cells through a hetero-octameric Gn/Gc spike complex that mediate receptor binding and subsequent virus-cell membrane fusion. Membrane fusion is triggered by exposure of Gc to mildly acidic pH in endosomes, inducing an irreversible conformational change into a post-fusion Gc-homotrimer (Gc3), characterized by its high stability, resistance to SDS and enzymatic digestion. While for other class II viral fusion proteins such as those from alpha- and flaviviruses, the biogenesis, priming and protection mechanisms have been well described to protect them from mildly acidic pH in the secretory pathway, such process in hantavirus fusion protein remains poorly described.

Materials and Methods: RER and Golgi enriched fractions were obtained from HEK293FT expressing ANDV GPC through calcium chloride precipitation and sucrose gradients, respectively. Gc from both fractions were tested to low pH sensitivity. Co-localization of a quaternary epitope of Gn/Gc with Golgi and RER was assessed by

fluorescent microscopy.

Results: We found that Gc in RER-enriched fractions undergo a stable post-fusion Gc-homotrimer upon acidification with high responsiveness to low pH. In contrast, in the Golgi-enriched fractions, Gc shows a reduced post-fusion Gc-homotrimer induction, suggesting a partial protection of Gn/Gc in this compartment. Fluorescent microscopy showed that the quaternary epitope, previously described to recognize Gn/Gc in the mature spike, shows punctate structures and a pattern that co-localize with Golgi, but not with RER. A short pulse with an acidic medium result in loss of the punctate structures, however, co-localization with Golgi remains intact.

Discussion: Evidence suggests that Gn/Gc organize in a structure capable of inducing a post-fusion Gc-homotrimer at RER, however it seems to not correspond to the mature structure of Gn/Gc at the spike. At Golgi, we observed that Gn/Gc reach an organization like the presented as mature spike, however, Gc is at least partially protected to low-pH activation in comparison to mature spike.

Acknowledgment: FONDECYT 1221811, FONDEF IDeA TA24I10051 and Centro Ciencia & Vida CTE Basal FB210008.

P73. The endogenous parvoviral derived protein DeRep inhibits rAAV2 replication. Isabel Fuentealba-Astudillo (i.fuentealbaastudill@uandresbello.edu), Pablo Lobos-Ávila, Javiera Bastías and Gloria Arriagada. Constantine-Paton Research Laboratory, Instituto de Ciencias Biomédicas, Universidad Andrés Bello, Santiago, Chile.

Introduction: Endogenous viral elements (EVEs) are genomic sequences derived from viruses. EVEs arise when infection of germline cells results in virus-derived DNA sequences being incorporated into chromosomes and inherited as host alleles. In *Octodon degus* the parvoviral endogenous element *EPV-Dependo.43-Odegus* encodes the protein DeRep. The DeRep mRNA allow the expression of two isoforms DeRep of 508 amino acids, and DeRepS of 386 amino acids. We have previously shown that when express in mice cells, DeRep has antiviral activity against the prototype parvovirus Minus Virus of Mice (MVM). However, it is unknown whether both isoforms exhibit antiviral activity against other parvoviruses, such as adeno-associated viruses (AAVs). AAVs are widely use as viral vector for gene therapy, but recently AAV2 was associated to an outbreak of hepatitis in children. Here, we evaluate the antiviral activity of the DeRep and DeRepS proteins against AAV2.

Materials and Methods: Using lentiviral vectors HEK293T cell expressing FLAG-DeRep and FLAG-DeRepS in a stable manner were generated. Protein expression was confirmed by western blot. Control and DeRep expressing cells were challenge with rAAV2-GFP and infection was quantified by detecting the GFP reporter protein. To analyze DeRep involvement on AAV2 genome replication, the cells were transfected with the plasmids required for rAAV2 production, and packaged viral genomes were quantified by qPCR.

Results: We found that DeRep does not affect rAAV2-GFP infection, but both FLAG-DeRep and FLAG-DeRepS expressing cells significantly reduced AAV2 genome production compared to the control cell line.

Discussion: These results suggest that DeRep plays a protective role against parvoviral infections, including those caused by AAVs at the viral at the genome replication or later steps of the replication cycle. It is necessary to investigate the mechanism through which these proteins exert antiviral activity to understand their potential for the development of therapeutic applications.

Acknowledgements: FONDECYT 1220480

P74. Inhibition of mitochondrial $\text{Na}^+/\text{Ca}^{2+}/\text{Li}^+$ exchanger NCLX alters tumorigenic traits and ER-mitochondria contact sites in MDA-MB-231 cells. Donato Garrido-Patiño^{1,2} (donato.p.garrido@gmail.com), Camila Quezada-Gutiérrez^{1,2,5}, Ulises Ahumada-Castro^{1,2}, Andrea Puebla-Huerta^{1,2}, Pablo Morgado-Cáceres^{1,2}, Antonio Martínez-Ruiz⁵ and J. César Cárdenas^{1,2,3,4}.

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Introduction: Triple-negative breast cancer (TNBC) is characterized by aggressive behavior, therapeutic resistance, and high recurrence rates. Tumorigenesis is supported by mitochondrial metabolism and calcium fluxes, which regulate key cellular processes. In the mitochondrial matrix, calcium uptake is mediated by the mitochondrial calcium uniporter complex (MCU), and extrusion primarily through the mitochondrial Na⁺/Ca²⁺/Li⁺ exchanger (NCLX). This calcium originates mainly from the endoplasmic reticulum (ER) via mitochondria–ER contact sites (MERCs).

While altered mitochondrial calcium uptake is frequently studied in cancer, changes in calcium extrusion remain underexplored. It has been suggested that NCLX regulates mitochondrial calcium uptake and may exert dual effects on tumorigenic traits. We aimed to investigate how impaired mitochondrial calcium extrusion affects calcium homeostasis and TNBC cell behavior.

Materials and Methods: MDA-MB-231 cells were treated with NCLX inhibitors CGP-37157, ITH-12575, or siRNA against NCLX for 24–48 h. Migration was assessed using gap closure and transwell assays. Epithelial-to-mesenchymal transition (EMT) and MERCs-related proteins were analyzed by western blot. mRNA levels of EMT, pluripotency, and matrix remodeling markers were measured by RT-qPCR. Mitochondrial respiration was evaluated using Seahorse XF, and calcium fluxes with Fluo-4 and Rhod-2 probes. MERCs colocalization was assessed using KDEL-BFP and MitoTracker Red.

Results: NCLX inhibition or knockdown reduced cell migration but increased transcription of pluripotency markers and metalloproteinases. Decreased mitochondrial calcium uptake, MERCs adaptor proteins, and ER–mitochondria colocalization were observed, with minimal impact on mitochondrial function.

Discussion: NCLX inhibition modulates TNBC cell behavior, exhibiting both anti- and pro-tumorigenic effects. Reduced mitochondrial calcium uptake and MERCs disruption may serve as an adaptive mechanism to avoid calcium overload when extrusion is impaired, preserving mitochondrial function.

Acknowledgment: FONDECYT N° 1240807 and FONDAP N° 15150012.

P75. Epitranscriptomic modifications as regulators of HIV-1 replication in human microglia. Sebastian Giraldo-Ocampo^{1,2,3} (sebastian.giraldo@ug.uchile.cl), Catarina Ananias^{1,2,3}, Aracelly Gaete-Argel^{1,2,3}, Cecilia Rojas-Fuentes^{1,2,3}, Camila Ortega-Orellana^{1,2,3}, Delia Lopez^{1,2,3}, Fernando Valiente-Echeverría^{1,2,3} and Ricardo Soto-Rifo^{1,2,3}.

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Introduction: Previous studies have demonstrated that RNA modifications including m6A, m5C, and ac4C decorate the HIV-1 full-length RNA playing crucial roles in viral replication in CD4⁺ T lymphocytes but also in HeLa and HEK293T cells. As expected, these RNA modifications have been shown as important regulators of viral RNA metabolism from the Rev-mediated nuclear export to efficient Gag protein synthesis, and viral genomic RNA packaging. However, the function of these epitranscriptomic modifications during HIV-1 replication in myeloid cells such as microglia, the primary target for the virus in the central nervous system, remains unclear. In this work we aimed at determining the role of m6A, m5C, and ac4C RNA modifications during HIV-1 replication in human microglia.

Materials and Methods: siRNA targeting the mRNA of the proteins that add the modifications m6A (METTL3/14), m5C (NSUN2), and ac4C (NAT10) was used in C20 cells (human microglia cell line) to evaluate the importance of these chemical modifications during HIV-1 replication.

Results: We observed a significant decrease in the intracellular and extracellular levels of viral genomic RNA and Gag protein in METTL3/14 KD cells compared to control cells in an effect exerted by the m6A readers YTHDF1-3. Moreover, although intracellular levels of viral RNA and Gag protein levels remained unchanged upon NSUN2 and NAT10 silencing (contrary to what was previously reported for T-lymphocytes), an important decrease in the extracellular viral copy number output was observed, indicating an effect for m5C and/or ac4C on the packaging or release of the genomic RNA.

Discussion: Together, this work demonstrates the differential role of RNA modifications during the HIV-1 replication cycle in human microglia with m6A regulating the intracellular metabolism of the viral RNA and m5C and ac4C probably acting as important factors for the proper release of viral RNA from the infected cell.

Acknowledgment: Fondecyt N° 1230102, Anillo ATE220016, ANID-ICM ICN2021_045, and ANID PhD fellowship N° 21230935.

P76. Evaluation of Binding Affinity of a Lactadherin-Blocking Aptamer through Mass Photometry.

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Introduction: Triple-negative breast cancer (TNBC) lacks effective therapeutic targets, representing a major clinical challenge. In this context, Lactadherin, a secreted glycoprotein overexpressed in breast tumor cells, has been implicated in promoting metastatic capacities. Previous experiments in our laboratory demonstrated that immunoblockade of Lactadherin with commercial antibodies reduces metastasis, highlighting its potential as a therapeutic target. In this context, the aptamers, short oligonucleotides, emerge as an attractive alternative to antibodies due to their lower production cost, structural stability, and minimal immunogenicity. This study aimed to assess the affinity of anti-Lactadherin aptamers using Mass Photometry (MP), a label-free technique capable of quantifying molecular interactions under native conditions.

Materials and Methods: Recombinant Lactadherin (2 nM) was used as the target, and two aptamers (Apt-2 and Apt-4) were tested. Binding curves were generated by varying aptamer concentrations from 20 nM to 0.02 nM. Measurements were performed in triplicate at 37 °C. MP distributions were analyzed to quantify free and bound species, enabling estimation of dissociation constants (Kd).

Results: Both aptamers displayed high affinity for Lactadherin, with dissociation constants in the 10⁻¹⁰-10⁻¹¹ M range. These values are comparable to those reported for therapeutic antibodies used in breast cancer, such as trastuzumab against HER2 (10⁻¹⁰-10⁻⁹ M). This indicates that the aptamers achieve blocking capacities equivalent to or exceeding commercial antibodies. Furthermore, MP confirmed the homogeneity of protein-aptamer complexes and excluded aggregation, supporting the robustness of this approach relative to conventional techniques such as microscale thermophoresis.

Discussion: Mass Photometry was established as a reliable method to characterize aptamer-protein. Subnanomolar-nanomolar affinities of Apt-2 and Apt-4 validate their potential as therapeutic candidates for TNBC, while demonstrating MP as a powerful, label-free tool for affinity determination in biomolecular research.

Acknowledgment: FONDEF N°ID25i10020, FONDECYT N°1221031, FONDEQUIP EQM-240012.

P77. Epigenetic modulation of macrophage polarization in colorectal cancer. Yanitza Gutierrez-Monsalve (ygutierrezm@magister.ucsc.cl), Matias I. Hepp.

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Introduction: Colorectal cancer (CRC) is one of the most prevalent and deadly malignancies worldwide. Its tumor microenvironment (TME) is characterized by extensive immune cell infiltration, with macrophages standing out due to their remarkable plasticity. Depending on environmental stimuli, macrophages polarize into either the M1 phenotype, associated with antitumor activity, or the M2 phenotype, linked to tumor progression and tissue repair. The transcription factor STAT3, frequently activated in CRC, drives polarization toward the M2 phenotype. Recent evidence suggests that STAT3 activation can be epigenetically modulated by inhibiting histone deacetylase 6 (HDAC6), opening new therapeutic opportunities to reprogram macrophage function within the TME. The aim of

this study was to evaluate whether selective HDAC6 inhibition modulates macrophage polarization by suppressing STAT3 activation and promoting an M1-like antitumor profile, potentially affecting cellular responses in CRC.

Materials and Methods: THP-1 monocytes were differentiated into macrophages (M0) using PMA and subsequently polarized into M1 or M2 phenotypes. Cells were then treated with NextA, a selective HDAC6 inhibitor. STAT3 expression and activation, as well as phenotypic-specific markers, were subsequently assessed. Cellular responses in CRC cells (HCT-116 and HT-29) were also evaluated by exposure to M1 or M2-conditioned medium, with or without NextA treatment. Different responses were analyzed by Western blot, RT-qPCR, and immunofluorescence.

Results: Treatment with NextA suppressed M2 polarization by reducing STAT3 activation, and a decrease in M2-like markers was observed, suggesting a shift towards an antitumor phenotype. Being confirmed by the deregulation of some immunomodulators in CRC cells exposed to conditioned medium (M1 or M2).

Discussion: These findings demonstrate that HDAC6 inhibition impairs STAT3-dependent M2 polarization and that affect cellular response in CRC cells, supporting HDAC6 as a promising therapeutic target for reprogramming macrophage function in CRC.

Acknowledgment: Project DIReg 11/2025.

P78. Characterization of two novel cell death-inducing proteins identified through whole-secretome protein structural analyses in the fungal phytopathogen *Botrytis cinerea*. Valentina Hadler^{1,3} (hadlervalentina@gmail.com), Patricio Sánchez^{1,2,3}, Romina V. Sepúlveda^{4,5}, and Paulo Canessa^{1,3}.

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Introduction: *Botrytis cinerea* is a necrotrophic phytopathogen characterized by gray mold. Its ability to infect plants, including crops of agricultural and economic importance, mainly depends on a subset of virulence factors known as Cell death-inducing proteins (CDIPs), which are a subgroup of small, extracellular, cysteine-rich effector proteins secreted during fungal infection. Some of these proteins can trigger defense responses in plants. Several studies suggest the existence of a broad range of CDIPs that have yet to be identified.

Materials and Methods: To this end, 18 candidates were selected based on their structural similarity to experimentally validated CDIPs. Then, to assess their ability to cause lesions in plants, these proteins were heterologously expressed in *Escherichia coli*. The cell-free culture supernatants, which contained the proteins of interest, were infiltrated into *Nicotiana benthamiana* leaves. For those that showed lesion development, the expression levels of key plant defense genes were measured using RT-qPCR.

Results: Based on the initial screening, the infiltration of two proteins, Bcin04g00470 and Bcin06g00540, produced visible lesions of varying size, edge, and texture in *N. benthamiana* leaves, indicating that the identified proteins can indeed induce cell death in plants. In fact, the infiltrated Bcin06g00540 protein triggers the upregulation of genes associated with the plant's cell-death defense response known as hypersensitive response (HR).

Discussion: The identification of new CDIPs through computational protein structural screening led to the discovery of at least two proteins that cause cell death symptoms in *N. benthamiana*, one of which exhibits a specific mode of action that could enhance fungal virulence.

Acknowledgment: This research was funded by ANID-Ph.D. national scholarships 2023-21232227 to P.S. and by the ANID-FONDECYT grant number 1240742 to P.C.

P79. The cellular RNA helicase DDX3X binds directly to Zika virus 5' untranslated region to promote viral translation. Tomás Hernández-Díaz^{1,2,3} (tomas.hernandez@ug.uchile.cl), Alonso González-Poblete^{1,2,3}, Sebastián Giraldo-Ocampo^{1,2,3}, Cecilia Rojas-Fuentes^{1,2,3}, Aarón Oyarzún-Arrau^{1,2,3}, Fernando Carrasco-Galvéz^{1,2,3}, Mónica L. Acevedo^{1,2,3}, Delia López-Palma^{1,2,3}, Aracelly Gaete-Argel^{1,2,3}, Barbara Rojas-Araya^{2,4}, Marcelo López-Lastra^{2,4},

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Introduction: Zika virus (ZIKV) is a mosquito-borne virus considered a global threat to public health due to its pandemic potential and its ability to cause neurological defects in newborns and adults. During ZIKV infection, ZIKV uses host proteins to avoid/abrogate cellular defenses to favor its own replication. Indeed, different RNA viruses including HIV-1, dengue virus and Hepatitis C virus usurp immune response-related cellular factors, such as the RNA helicase DDX3X, to avoid the innate immune response and promote viral replication. DDX3X is characterized as a cellular ATP-dependent RNA helicase that binds to cellular and viral RNA with secondary structures close to the cap at 5'UTR to promote protein synthesis.

The aim of this work was to determine the mechanism by which DDX3X participates in viral replication of ZIKV and determine how the interaction between DDX3X and viral RNA occurs.

Materials and Methods: The C20 human microglia cell line was used to performed infections, where we evaluated interaction between DDX3X and ZIKV RNA by RNA-immunoprecipitation and confocal microscopy. Computational modeling of DDX3X and ZIKV RNA 5'UTR interaction was performed with molecular dynamics using HDOCK server. For *in vitro* assays, we used recombinant DDX3X (132-607) expressed in *E. coli* and ZIKV 5'UTR was synthesized by *in vitro* transcription.

Results: We observed that DDX3X interacts with viral RNA during infection in human microglia. Three-dimensional molecular dynamic of ZIKV 5'UTR reveals a conserved conformation with secondary structures, and simulation of interaction with DDX3X shown an interaction with 5'UTR in CCR1 region. These results were validated by electrophoretic mobility shift assay and microscale thermophoresis where DDX3X binds directly to 5'UTR. Moreover, this binding stimulates DDX3X ATPase activity. Finally, using a ZIKV-derived reporter mRNA, we show that DDX3X promotes viral RNA translation.

Discussion: Together, these results indicate that DDX3X binds to ZIKV RNA at its 5'UTR to promote viral protein synthesis.

Acknowledgment: ANID-FONDECYT Program, grants N° 1230102 to R.S.R.; N° 1251218 to F.V.E.; N° 1210736 to M.L.-L. and N° 11230976 to M.Z.-B. ANID-ICM, ICN 2021_045 to R.S.R., F.V.E. and M.L.L., Anillo Grant ATE220016 to R.S.R. and F.V.E.

P80. Characterization of baseline mitochondrial differences in cardiomyocytes from male and female neonatal rats and their impact on the response to anthracycline damage. Magdalena Jiménez^{1,2} (magdalena.jimenez@uc.cl), Wileidy Gomez^{1,2}, Ingrid Oyarzun^{1,2}, Georhan Mancilla^{1,2,3,4}, Pablo Castro^{1,2}, Hugo Verdejo^{1,2}, Clara Quiroga^{1,2}.

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Introduction: Sex-related differences are well documented in cardiovascular diseases. The studies have been performed mainly in adult rat and mouse animal models, and are often attributed to sex hormones, signaling pathways, and differential gene expression. Due to the sexual dimorphism observed at cardiovascular and physiological levels, we hypothesized that there are baseline differences between the two sexes that could influence various cellular processes, such as energy metabolism, signaling, stress response, and drug metabolism. Considering the central role of mitochondria in cardiomyocyte function, we initially decided to characterize possible differences in the expression and levels of certain mitochondrial proteins, as well as to assess their impact

on the response to doxorubicin, a cardiotoxic anthracycline known to induce senescence and cell death in a dose-dependent manner.

Materials and Methods: Cardiomyocytes were isolated from male and female neonatal Sprague-Dawley rats. Gene expression and protein levels were determined using methods such as qPCR and Western blot, focusing on several genes and proteins involved in mitochondrial dynamics and function (e.g., PGC-1 α , HSP70m, TOM20, Mic19, Mic60, MTFP1, among others). Cells were treated with 50 nM doxorubicin for 72 hours to induce stress.

Results: Although no statistically significant differences were observed, trends suggested higher expression of mitochondrial genes in female-derived cells, which was not replicated at the protein level. Preliminary findings also indicated possible sex-dependent responses to doxorubicin. For instance, Mic19 showed a trend toward increase in male-derived cardiomyocytes (p=0.06).

Discussion: These results point to subtle sex-related differences in the stabilization and half-life of mitochondrial proteins, which may influence cardiomyocyte responses to stress. Further studies are needed to confirm these observations and clarify their physiological relevance in cardiovascular biology.

Acknowledgment: PUENTE-UC 2024-7 (CQ), FONDAP-1523A000 (CQ-PC-HV).

81. Smart XY Motion and AI-Driven Particle Tracking for Next-Gen Force Spectroscopy on DNA. Vicente Ladrix^{1,2} (ladrixvicente@gmail.com), Tomás Hermosilla^{1,2}, Felipe Urbina³, Jaime Andrés Rivas-Pardo².

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Introduction: Single-molecule force spectroscopy (SMFS) provides unique insights into DNA–ligand interactions and biomolecular mechanics. Magnetic tweezers (MT), a key SMFS technique, enable real-time force and displacement measurements but face persistent challenges in achieving high-precision micrometer positioning and reliable bead tracking. To address these limitations, we developed an automated MT platform integrating a motorized XY motion system with intelligent particle detection.

Materials and Methods: The microscope was built with optical, electronic, and 3D-printed components to achieve micrometric precision using NEMA stepper motors. For automation, object detection was implemented with the YOLO deep learning algorithm trained on microscopy images, and particle tracking/vibration analysis employed the Kanade–Luca’s method via Python and OpenCV. Standard biotin–streptavidin DNA tethers and paramagnetic beads were used to validate the system in force spectroscopy assays.

Results: The platform successfully integrated high-precision XY control with automated particle detection and tracking. The software modules enabled real-time identification and monitoring of beads, reducing operator bias and advancing towards full instrument automation. The system was robust, reproducible, and modular, supporting seamless hardware–software integration.

Discussion: By combining motorized control with AI-based detection, this work demonstrates a significant step towards automated, high-throughput single-molecule force spectroscopy. Beyond technical advances, the platform provides a versatile foundation for probing DNA mechanics and for systematic studies of biomolecular interactions, including drug–DNA binding and protein–DNA dynamics.

Acknowledgment: FONDECYT 1221064

P82. Expression of a therapeutic nanobody in Black soldier fly (*Hermetia illucens*) larvae. Daniela Leiva Carvajal (danielaleiva@bybug.io), Valentina Varela Silva, Jorge Caballero González, Nicolás Órdenes-Aenishanslins, Valentina Muñoz Zapata, José del Solar Bou, Daniel Troncoso-Espinosa, Clara A. Solari.

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Introduction: The increasing demand for biopharmaceuticals, such as single-domain antibodies (VHHs), underscores the need for innovative, scalable, and cost-effective production platforms. ByBug has recently developed a promising platform for therapeutic protein expression in Black Soldier Fly (BSF) (*Hermetia illucens*). This study aimed to validate a methodology for generating transgenic BSF larvae capable of expressing a therapeutic VHH for application in the poultry industry.

Materials and Methods: Insect rearing was conducted in a climate-controlled facility, and fed a standardized diet to minimize biological variability. BSF embryos were microinjected with a custom expression vector containing the codon optimized sequence of the nanobody and a fluorescent marker. Surviving larvae were subjected to a two-step screening protocol: fluorescence stereomicroscopy, followed by PCR with VHH-specific primers. Larva homogenates were then subjected to downstream protein detection and quantification steps including affinity chromatography, SDS-PAGE and ELISA.

Results: PCR confirmed the presence of the transgene in fluorescent larvae. The identity of the inserted fragment was further verified through Sanger sequencing. The overall transformation rate was 0,03%, calculated as the number of PCR-positive larvae relative to the total number of microinjected embryos. The positivity rate was 6,6% of the survival larvae. The VHH was detected in larvae protein extracts through ELISA with a yield of 2mg/kg of biomass.

Discussion: These results validate the pipeline for generating and characterizing recombinant *H. illucens* larvae. This positions ByBug Synthetic® as a viable and efficient platform for the production of nanobodies and other high-value proteins, opening new opportunities in pharmaceutical biotechnology.

Acknowledgment: Startup Ciencia 2024. ANID. SUC240043

P83. Trigger Factor as a modulator of the Structural Dynamics of FoxP1 FKH domain. Jesús Lira Gerardo (jesus.lira@ug.uchile.cl), Elias Manriquez, Camila Jorquera, Mauricio Báez, Exequiel Medina. Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.

Introduction: Human transcription factors FoxP are proteins involved in various aspects of cellular development, such as language and immune response. These proteins contain a highly conserved DNA-binding domain (FKH), which dimerize via three-dimensional domain-swapping (3D-DS). Several studies have shown that the presence of disordered regions affect the FKH's dimerization properties, suggesting their importance in the transcriptional function of these proteins. Trigger Factor (TF), a chaperone involved in proper protein folding in *Escherichia coli*, recognizes exposed hydrophobic regions in proteins, thus facilitating the correct folding. However, it is unknown how chaperones affect complex folding mechanisms such as 3D-DS.

Materials and Methods: Single-cysteine mutants of the FKH domain of FoxP1 (S57C and V78C) were generated and purified to compare the effect of TF on helices H3 and H5, respectively, in the monomer. These mutants were labeled with Bodipy as a probe, and the structural flexibility was investigated using fluorescence anisotropy at the single-molecule level. Furthermore, a double mutant (S57C/V78C) was prepared single-molecule FRET (smFRET) experiments to probe conformational dynamics between helices H3 and H5.

Results: Single-molecule anisotropy revealed that the flexibility of the FKH domain is heterogeneous, with distinct behavior observed for helix H3 (S57C) and helix H5 (V78C). The presence of the chaperone TF induces a global stabilization of a highly flexible intermediate. Preliminary smFRET data on the S57C/V78C mutant suggest that TF modulates the conformational landscape of the FKH monomer.

Discussion: Together, these results suggest that TF directly interacts with the FKH monomer, modulating its structural dynamics. By stabilizing a flexible and open intermediate state, as indicated by both anisotropy and smFRET, this chaperone could modulate the equilibrium of the 3D-DS, thereby affecting the gene-expression activity of FoxP1.

Acknowledgment: Fondecyt 1251879; Fondecyt 1231276; Fondecyt EQM200202

P84. Analysis of the antiviral activity of Rep-derived endogenous parvoviral elements. Pablo Lobos-Ávila (p.lobosvila@uandresbello.edu), Felipe Galleguillos, Camila Osega-Ríos, Fernando J Bustos and Gloria Arriagada. Constantine-Paton Research Laboratory, Instituto de Ciencias Biomédicas, Universidad Andrés Bello, Santiago, Chile.

Introduction: Endogenous parvoviral elements (EPVs) represent ancestral parvoviral infections in a hosts germ line present in extant species. In *Octodon degus*, *Chinchilla lanigera*, and *Oryctolagus cuniculus*, we identified EPVs that encode Rep-derived proteins, named DeRep, CLanRep, and OcRep. *In vitro*, DeRep has antiviral activity against the Minute Virus of Mice (MVM) and localizes to the cell nucleus. In *O. degus*, we also identified

a smaller protein (DeRepS), corresponding to the C-terminal portion of DeRep. Here, we aim to evaluate which of these *Rep*-derived proteins protect cells against MVM infections.

Materials and Methods: Stable NIH3T3 cell lines expressing FLAG-DeRep, FLAG-DeRepS, FLAG-DeRep Δ S (lacking the expression of DeRepS), FLAG-DeRep Δ NLS (mutated in its nuclear localization signal), FLAG-CLanRep, and FLAG-OcRep were generated. Protein expression and localization were analyzed by western blot and immunofluorescence. Cells were challenged with MVM, surviving cells were measured 5 days post-infection.

Results: All proteins, except FLAG-DeRep Δ NLS and FLAG-OcRep, localized in the nucleus. The nuclear located proteins FLAG-DeRep, FLAG-DeRepS, FLAG-DeRep Δ S and FLAG-CLanRep protect cells against MVM infection. On the contrary FLAG-OcRep does not present antiviral activity. Surprisingly, FLAG-DeRep Δ NLS only protect cells infected with low doses of MVM. By immunofluorescence we observed DeRep Δ NLS in the nucleus upon MVM infection.

Discussion: EPVs derived-proteins found in chinchilla and degu, can block MVM infection. In degu two proteins were previously found, DeRep and DeRepS, each one can independently protect cells against MVM infection. Since DeRepS lacks the DNA binding domain of DeRep, our results suggest that an interaction with viral proteins could mediate the antiviral activity. This hypothesis is supported by the protection observed in DeRep Δ NLS expressing cells.

Acknowledgment: FONDECYT 1220480

P85. Prior asymptomatic herpes simplex virus type 1 infection causes more severe sepsis and increased blood-brain barrier permeability in the endotoxemic mouse model. Dayesi López-Hernández (dlopez9@uc.cl)^{1,2}, Rodrigo Reyes-Ramírez^{1,2}, Areli J. Navarro^{1,2}, Luisa F. Duarte^{1,4}, Javier Carbone-Schellman^{1,4}, Susan M. Bueno^{1,2}, Leandro J. Carreño^{1,5}, Alexis M. Kalergis^{1,2,6}, Claudia A. Riedel^{1,3}, Felipe Simon^{1,3}, Pablo A. González^{1,2}.

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Introduction: Sepsis is a life-threatening condition which can lead to sepsis-associated encephalopathy and multiple organ dysfunction syndrome due to a dysregulated host response to infection. While bacterial pathogens and endotoxins are primary triggers, host-related factors such as age, genetics, and comorbidities significantly influence sepsis outcomes. Recent studies suggest that latent viral infections, including herpes simplex virus type 1 (HSV-1), may exacerbate sepsis progression, nonetheless, a relationship between prior HSV-1 infection and sepsis severity remains unclear. In this study, we evaluated the impact of previous asymptomatic HSV-1 infection on the onset and severity of sepsis upon endotoxemia in a mouse model.

Materials and methods: C57BL/6 male mice were infected intranasally with HSV-1 or mock-treated as controls. Both virus-infected and uninfected mice were subjected to endotoxemia via intraperitoneal LPS injection 30-35 days post-infection. Survival and sepsis clinical score were evaluated post-endotoxemia induction. We also measured biochemical parameters in the blood, as well as cytokine mRNA levels and viral loads in the brain and liver tissues by qPCR. Furthermore, blood-brain barrier integrity was evaluated using an Evans blue dye exclusion test to assess blood vessel permeability.

Results: We observed significantly more severe sepsis and lower survival rates in septic mice previously infected with HSV-1. Furthermore, infected animals displayed significantly increased levels of alanine aminotransferase (ALT) and bilirubin levels in the plasma. Moreover, previously infected animals underwent endotoxemia, with increased levels of viral genome copies compared with mice that were previously infected and challenged with vehicle alone. Finally, we observed increased permeability of the blood-brain barrier in virus-infected and endotoxemic mice compared to control groups.

Discussion: Our results demonstrated that prior asymptomatic HSV-1 infection exacerbates sepsis severity and the likelihood of increased risk of neuroinflammation.

Acknowledgments: Millennium Institute on Immunology and Immunotherapy ICN2021_045, FONDECYT 1240971, FONDECYT 1241072 and ANID Scholarship 21202257.

P86. Non-Catalytic roles of epigenetic regulators in mouse Embryonic Stem Cells. Constanza Mardones-Molina¹ (c.mardonesmolina3@uandresbello.edu), Gustavo Jara¹, Sebastián Echeverría-Araya, Bruno Soto-Moraga Anjana Rao², Hugo Sepúlveda^{1,2}.

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Introduction: The several chromatin-modifying enzymes also play non-catalytic regulatory roles. Prominent examples include MLL3/4 (COMPASS), UTX and LSD1, which, in addition to their enzymatic activity, contribute to epigenome organization through non-catalytic functions. We propose OGT, which is essential for cell survival, not only adds O-GlcNAc to nuclear proteins but also modulates transcription and DNA modifications through scaffold functions. These findings highlight the need to determine the extent to which epigenetic effects depend on catalytic or non-catalytic functions, as well as to identify novel enzymes involved.

Methodology: Using mESCs carrying an inducible genetic system to remove OGT (*Ogt-iKO*), we assessed changes on gene expression and 5mC/5hmC patterns by RNA-seq and 6base-seq, respectively, comparing them against mESC where only the catalytic function of OGT was abolished (OSMI4). Similarly, we compare our results with datasets from chromatin regulators known to have functions independent of their catalytic activities (MLL3/4;UTX;LSD1). Hi-C data from WT-mESC was applied to determine heterochromatic and euchromatic compartments, and ChIP-seq mapped chromatin regulator enrichment. These approaches allowed us to determine the impact of both catalytic and non-catalytic functions on chromatin organization comprehensively.

Results: Inhibition of catalytic activity of chromatin regulators does not fully recapitulate the chromatin changes seen after complete depletion. For OGT, catalytic and non-catalytic models differentially influence transcription and DNA modifications at promoters, enhancers, and transposable elements. These findings highlight how epigenetic regulators shape chromatin architecture and transcription in mESCs through both catalytic and non-catalytic functions.

Discussion: The non-canonical roles of epigenetic regulators affect transcription, DNA modifications, and chromatin organization in mESCs. These non-catalytic functions represent an important new dimension that we need to integrate in our understanding of epigenetic regulation and in the development of future therapies.

Acknowledgements: The PEW Charitable Trusts, US.

P87. ProteoAR: Protein Structure Book with Augmented Reality. Martínez-Oyanedel J. (jmartine@udec.cl) and Bruna Cofre C.

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Introduction: Most Biochemistry courses address the structural organization of proteins-from primary to quaternary levels-in order to establish the relationship between structure and function. However, one of the main challenges in teaching this topic lies in the predominantly two-dimensional representations found in textbooks, websites, and lecture materials, which often hinder students' ability to conceptualize the three-dimensional nature of macromolecules. Augmented Reality (AR) offers an accessible solution, allowing students to visualize and interact with 3D structures of biomolecules directly on their mobile devices. In this project, an *Augmented Reality Protein Structure Book* was created, along with a mobile application that enables students to view and manipulate 3D molecular models in real space, enhancing the understanding of protein architecture.

Materials and Methods: A book focused on protein structure was developed, emphasizing the use of diagrams and visual representations. Molecular images were generated using PyMOL 3.0, based on atomic coordinates retrieved from the Protein Data Bank (PDB). For each image, corresponding 3D AR objects and markers were created. A mobile application was developed using Unity, allowing users to scan the markers in the book and visualize the 3D structures through their phones. To assess the educational impact and user perception, students from two General Biochemistry courses were

invited to use the book and app as supplementary study material. Pre- and post-intervention questionnaires were administered to evaluate both the pedagogical effectiveness of the material and students' familiarity with and attitudes toward Augmented Reality in education.

Results: In each course, over 50% of the students opted to participate in the use of the book and the mobile app. Preliminary results have been encouraging: a significant portion of students had no prior experience with AR, yet responded positively to its use in the learning environment. The results of the pre- and post-test surveys indicate a notable increase in engagement and understanding. Detailed findings on student perceptions and learning outcomes will be presented.

Acknowledgment: Proyecto Colabora I3D- 133

P88. Loss of *Sall2* in organoid-derived colorectal cancer tumors is associated with decreased survival rates and stromal remodeling toward a pro-invasive phenotype. [Paula Medina Caro¹ \(pmedina2018@udec.cl\)](mailto:pmedina2018@udec.cl), Araceli Castro Nahuelcura¹, Diego Benítez-Riquelme¹, Manuel Mastel², Aracelly Quiroz Lagos³, Antonia Nova González¹, Tomás Molina Gallardo¹, René Jackstadt², Ariel Castro¹, Roxana Pincheira¹.

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Introduction: Colorectal cancer (CRC) is a leading cause of cancer-related mortality worldwide. Its progression depends on genetic alterations and signals from the tumor microenvironment (TME). The *Sall2* transcription factor has been described as a tumor suppressor and is downregulated during CRC progression; however, its role in CRC remains poorly understood.

Materials and Methods: This study aimed to evaluate the contribution of *Sall2* loss to CRC progression and the TME through the generation of advanced CRC models based on CRISPR-Cas9- mediated genome editing of *Sall2* in AKP organoids (*Apc*^{-/-}, *Kras*^{V12}, *Trp53*^{-/-}), which were then injected into the distal colon of mice. Organoids with mutations in *Apc*, *Kras*, and *Trp53* (AKP), with or without *Sall2* expression (AKP vs. AKPS), were generated using CRISPR-Cas9. They were microinjected into the colonic mucosa of C57BL/6 mice using a site-directed colonoscopy. Kaplan-Meier curves were used to evaluate overall survival, and colonoscopy and histological techniques were used to evaluate CRC progression over time. Tumor stromal composition was assessed using H&E, Masson's Trichrome, Picro-Sirius Red, and immunohistochemistry.

Results: Murine CRC models injected with AKPS organoids showed significantly reduced survival compared to AKP controls, which correlated with more aggressive tumor growth and partial epithelial- to-mesenchymal transition. Histochemistry revealed that *Sall2* loss induced extensive stromal remodeling, characterized by an increased percentage of type I and III collagen and increased collagen fiber alignment, suggesting a more rigid and pro-invasive extracellular matrix associated with poor prognosis. These changes were consistent with the activation of cancer-associated fibroblasts or myofibroblasts and structural alterations that support tumor invasion.

Discussion: Together, these findings demonstrate that *Sall2* loss not only drives increased tumor aggressiveness and decreased survival but also remodels the TME by promoting a pro-tumorigenic stroma. Therefore, *Sall2* emerges as a central regulator of CRC progression, acting at both epithelial and stromal levels, and represents a potential therapeutic target for this disease.

Acknowledgment: FONDECYT 1241771, VRID DICA N°170/25

P89. Two Roads, Two Substrates: Tracing Divergent Pathways of Substrate Specificity Evolution. [Claudia Mella Hernández \(claudiamella@ug.uchile.cl\)](mailto:claudiamella@ug.uchile.cl), Victoria Guixé, and Victor Castro Fernández. Laboratorio de Bioquímica y Biología Molecular, Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile.

Introduction: In the Histidine Acid Phosphatase (HAP) family of *Enterobacteriales*, phytase and glucose-1-phosphatase (G1Pase) share the same catalytic mechanism but differ in substrate specificity: phytase hydrolyzes

myo-inositol hexakisphosphate (InsP₆), while G1Pase acts mainly on glucose-1-phosphate (G1P) with promiscuous activity toward InsP₆.

Materials and Methods: In this work, we performed Ancestral Sequence Reconstruction (ASR) to trace the evolutionary origin of the phytase activity by inferring: (i) the last common ancestor of *Enterobacterales* phytases (ancEnPhy), (ii) the common ancestor of phytases from α - and γ -proteobacteria (ancPhy) and (iii) the ancestor of both lineages (ancPhyG1P). These reconstructed enzymes were expressed, purified, and kinetically characterized.

Results: The ancPhyG1P ancestor, exhibited activity toward both G1P and InsP₆, although with lower catalytic efficiency than substrate-specific extant enzymes. In contrast, ancPhy and ancEnPhy, on the evolutionary path to modern phytases, displayed higher catalytic efficiency toward InsP₆ and reduced efficiency toward G1P, showing a shift from a promiscuous ancestor to specialized extant phytases.

Structural and evolutionary analyses identified two insertions (S α and S β) present in phytases but absent in G1Pase. To test their contribution to the appearance of phytase activity, we engineered two ancPhyG1P variants: a phytase-like mutant with the insertion of S β , and a G1Pase-like mutant by deleting the S α segment.

Deletion of S α led to a 2–3-fold increase in catalytic efficiency toward G1P, suggesting that specialization toward phytate emerged primarily by a trade-off involving reduced efficiency toward G1P. In contrast, insertion of S β alone did not significantly affect the kinetic parameters for InsP₆ or G1P compared to ancPhyG1P.

Discussion: These results show that the insertions of S α and S β alone are not sufficient to drive significant changes in substrate specificity within this family. Rather, they likely act in concert with other amino acid substitutions, contributing to the progressive optimization of phytase activity in the *Enterobacterales* lineage.

Acknowledgment: FONDECYT N° 1221667.

P90. The Role of Cellular Prion Protein (PrPc) in Cardiac Hypertrophy and Oxidative Stress in Cardiomyocytes. Evaristo Molina^{1,2} (evaristo.molina@ug.uchile.cl), Úrsula Zúñiga-Cuevas^{1,2}, Leslye Venegas-Zamora^{1,2}, Valentina Parra^{1,2,3}.

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Introduction: Cardiac hypertrophy is characterized by enlargement of the heart and alterations in several processes within cardiomyocytes, such as increased oxidative stress, which can ultimately progress to heart failure. The cellular prion protein (PrPc) has recently emerged as a novel biomarker of maladaptive cardiac hypertrophy. In cardiomyocytes treated with norepinephrine or angiotensin II, both hypertrophic markers and PrPc levels were increased. Furthermore, PrPc overexpression has been shown to reduce ischemia/reperfusion-induced cell death in mouse hearts, as well as to decrease the accumulation of reactive oxygen species, suggesting a cardioprotective role for this protein. Based on these findings, we hypothesized that PrPc regulates the hypertrophic response in cardiomyocytes through the modulation of the antioxidant response

Materials and Methods: Neonatal rat ventricular myocytes (NRVMs) were treated with norepinephrine (NE) for 48 hours. The hypertrophic response was assessed by measuring cell area and perimeter using immunofluorescence, as well as the expression of hypertrophic markers (ANP and BNP), antioxidant-related genes (Nrf2, CAT, SOD2), and PrPc by qPCR. In addition, PrPc expression was silenced using siRNA to evaluate its role in the same hypertrophic parameters. The presence of reactive oxygen species (ROS) was quantified using fluorescent probes analyzed by flow cytometry.

Results: Norepinephrine treatment effectively induced cardiomyocyte hypertrophy, as evidenced by increased cell area, perimeter, and expression of hypertrophic markers, including PrPc. Silencing PrPc further cell perimeter and hypertrophy marker expression. Moreover, PrPc knockdown led to increased expression of antioxidant response genes and elevated ROS levels.

Discussion: The upregulation of PrPc in norepinephrine-stimulated cardiomyocytes, together with the exacerbated hypertrophic and oxidative responses observed upon its knockdown, suggest that PrPc modulates the hypertrophic response. Collectively, these findings identify PrPc as a potential molecular target in the context of cardiovascular diseases.

Acknowledgment: This project is funded by ANID FONDECYT 1230195 (VP) and FONDAP 15130011 (VP).

P91. Potential Role of MicroRNAs in Extracellular Vesicles from Obese Women on Gallbladder Cancer Cells Phenotype. María Montenegro^{1,2} (maria.montenegro.r@ug.uchile.cl), Camilo Torrejón², Eva Hormazabal², Victoria Rojas², Lorena Oróstica².

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Introduction: Obesity is a chronic disease associated with metabolic, inflammatory, and hormonal alterations that could promote cancer progression. Gallbladder cancer (GBC) shows an alarming incidence in Chile, especially among women, being a highly metastatic and lethal disease. Preliminary evidence suggests that serum extracellular vesicles (EVs) from obese women (EV-OB) promote the invasion and migration of GBC cells. RNA-seq analyses revealed that the microRNAs let-7a-5p, let-7i-5p, and miR-148a-3p are significantly downregulated in EV-OB compared with EVs from normal-weight women (EV-NP). We performed an in-silico analysis to explore the potential role of these microRNAs on the aggressiveness of GBC cells.

Materials and Methods: A gene ontology-based bioinformatics analysis was performed to investigate the potential role of exosomal microRNAs downregulated in obesity. Sequences of let-7a-5p, let-7i-5p, and miR-148a-3p were retrieved from miRBase. Predicted target transcripts were identified using TargetScan, miRDB, and experimentally validated interactions from miRTarBase. Overlapping targets among the three databases were selected for further analysis. Functional enrichment was conducted with Enrichr (Gene Ontology Biological Process, Reactome, and KEGG). For gene sets with low coverage, results were complemented with Elsevier Pathways. Statistical significance was defined as FDR-adjusted $P < 0.05$ and fold enrichment > 2 .

Results: We identified 9 targets for let-7a-5p, 5 for let-7i-5p, and 70 for miR-148a-3p. Key genes included HMGA1, PTEN, MET, and CDKN1B. Enriched pathways such as PI3K-Akt, focal adhesion, and epithelial-mesenchymal transition (EMT) are strongly linked to invasion and migration.

Discussion: These findings suggest that downregulation of these microRNAs in obesity may activate pro-metastatic pathways, supporting their role as regulators of invasion and migration. HMGA1, PTEN, MET, and CDKN1B emerge as strong candidates for further validation in GBC. Next, we will restore the levels of these microRNAs in EVs from obese women through mimic transfection and evaluate their effects on GBC cell invasion and migration.

Acknowledgment: PAI-SIA ANID 77190041 and Internal Research Grant for Academic Women at UDP.

Sponsored by: Lorena Lobos

P92. Antiproliferative Evaluation of MOp3-Derived Lipopeptides Synthesized via SPPS and Ugi Multicomponent Reaction. Moreno Bernal Rachel¹; Zuñiga Arbalti Felipe²; Campos Delgado Jose³; Weber Paixao Marcio³; Jiménez Águila Claudio¹.

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Introduction: Lipopeptides are promising anticancer agents due to their ability to interact with biological membranes and selectively impair cancer cell viability. MOp3, a hydrophobic anionic antimicrobial peptide derived from *Moringa oleifera* seeds, provides an attractive scaffold for developing lipopeptide derivatives with improved biological activity.

Materials and Methods: In this study, we synthesized and characterized a series of MOp3-based lipopeptides incorporating lipid chains through solid-phase peptide synthesis (SPPS) combined with on-resin Ugi multicomponent reactions mediated by aminocatalysis. The resulting compounds were purified by preparative RP-HPLC, characterized by mass spectrometry. Their antiproliferative activity was evaluated in vitro against

colorectal (CACO), gastric (AGS), hepatocellular (HepG2), oral (HSC3), and breast (MCF-7) cancer cell lines, with human dermal fibroblasts (HDF) as a non-cancerous control.

Results: Among the tested compounds, SPPS8 and SPPS9 demonstrated the most potent antiproliferative effects, particularly against AGS and MCF-7 cells, while showing minimal cytotoxicity toward HDF cells, suggesting a favorable selectivity profile. The incorporation of α,ω -amino lipid modifications in these derivatives appears to enhance their activity, likely by facilitating interaction with and disruption of cancer cell membranes.

Discussions: These results underscore the potential of MOp3-derived lipopeptides as selective and effective anticancer agents and illustrate the utility of combining SPPS and Ugi reactions for generating structurally diverse bioactive molecules.

Acknowledgment: ANID FONDECYT 1221631; Brazilian funding agencies CNPq (Grant No. 380675/2023-4); INCT Catálise, Grants No 444061/ 2018-5; Universal Project 405052/2021-9 and CTAgroInsumos 406245/2022-3) and FAPESP (21/06099-5).

Sponsored by: Maximiliano Figueroa

P93. Codon Usage Bias and tRNA Dynamics Under UV Stress in the Atacama desert Cyanobacterium *Nodularia* TAL-12. Valentina Muñoz Madrid^{1,4} (Valentina.munoz.m@ug.uchile.cl), Jan Ortiz^{2,3,4}, Gabriel Peña^{2,3,4}, Alexandra Galetovic^{2,3,4}, Álvaro Glavic^{1,4}.

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Introduction: Although the genetic code is conserved, codon usage varies among organisms and contexts. It has been proposed that codons recognized by the most abundant tRNAs are translated more efficiently. This codon usage bias reflects selective pressures to optimize translational, and it can be dynamically modulated under stress.

Material and Methods: We investigated codon usage adaptation in the *Nodularia* TAL-12, a cyanobacterium that produces scytonemin pigment in response to UV radiation. Transcriptomes were generated after 0, 48, and 96 hours of UV exposure, assessing changes in gene expression and codon usage relative to the genome. Expression of tRNA-modifying enzymes was examined, and exploratory tRNA sequencing was performed to evaluate correlation between tRNA abundance and codon usage.

Results: Codons such as TTG (Phe), AAT (Asn) and CAG (Gln) altered their usage frequency under UV exposure. These changes were associated with biological processes related to stress response and DNA repair. Consistently, variations were detected in the expression of tRNA-modifying enzymes involved in decoding these codons, including members of Tsa complex (responsible for t⁶A37) or Mnm complex (responsible for mnm⁵U34). tRNA analyses revealed that tRNA abundance correlates more strongly with codon usage under control conditions than under UV treatment. Moreover, changes in the expression of certain tRNA species during UV irradiation correlated with the dynamics of codon usage of the codon they decode.

Discussion: Our results suggest that codon usage plasticity is part of the adaptive mechanisms mobilized by UV radiation and highlight a regulatory layer of translational response to environmental stress. tRNA availability and chemical modifications act in concert to fine-tune decoding efficiency. Thus, the interplay between codon bias and tRNA epitranscriptome emerges as a part of the mechanisms of adaptation to UV radiation.

Acknowledgment: ANID ICN2021_044, ANID FONDECYT 1231105 and ANID 21231552.

P94. Stress responses shape *Leptospirillum ferriphillum* metabolisms. Javiera Norambuena¹ (javiera.norambuena@upla.cl), Carlos Farkas², Agustín Rojas¹.

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Introduction: The genus *Leptospirillum* is one of the most renowned bioleaching organisms because it can dominate natural and industrial environments. Bioleaching environments are highly oxidizing and have millimolar

concentrations of metals, which can trigger reactive oxygen species (ROS) and lead to cell damage. This organism has a very special metabolism, it lacks canonical ROS detoxification systems (catalases and superoxide dismutases). Instead its genome encodes for alternative detoxification systems such as: rubrerythrin, periplasmic cytochrome c peroxidase, and a putative iron-dependent peroxidase (efeB) are present. It has been shown that *Leptospirillum ferriphillum* does not produce glutathione or any other known low molecular weight thiol, it does encode for a thioredoxin system. To the date, very little information is known about how this organism responds to ROS or disulfide stress, simulated by hydrogen peroxide and diamide, respectively.

The aim of this work was to determine how *L. ferriphillum* responds to ROS and disulfide stress.

Materials and Methods: *L. ferriphillum* DSM 14647 was grown in 882 media over night at 37°C, cultures were resuspended in fresh medium to an optical density of 600nm of 0,25 and cells were exposed or not for 30 min to 1mM H₂O₂ or 4 mM diamide. Cells were washed and RNA was extracted NucleoSpin RNA (Macherey-Nagel™), treated with RNase-free DNase (Invitrogen Turbo DNA-free kit) and sent for Next Generation Sequencing (Azenta, South Plainfield, NJ). Differential gene expression (DGE) by were obtained.

Results: assembly of flagella appears to be a common response between stresses. Oxidative responses varied among stresses and they appeared to be stress specific, same happens for carbon metabolism. Unspecific responses like activation of metal toxicity genes was observed under both stress

Discussion: Together, these data suggest that disulfide stress and oxidative stress trigger differential responses in this organism.

Acknowledgment: FONDECYT iniciación N°11240568

P95. OxLDL/DRP1 Signaling Links Mitochondrial Fission to EMT in Prostate Cancer Cells. Olivas Henríquez G (golivas2019@udec.cl), Durán A., Azocar S., Castillo M, Duprat F., Pampaloni P., Rivas Y., Sanzana J., González-Chavarría I.

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Introduction: Prostate cancer (PCa) treatment failure is mainly associated with late-stage diagnosis, when the disease is metastatic. Metastasis requires PCa cells to undergo epithelial–mesenchymal transition (EMT), a process in which epithelial traits are lost while mesenchymal features and migratory/invasive capacities are acquired. Increased mitochondrial fission has been described as a key event promoting EMT in several cancer types. Our group has shown that oxLDL/LOX-1 signaling promotes EMT in PCa. In cardiovascular diseases such as atherosclerosis, elevated oxLDL levels induce mitochondrial fission through DRP1 activation. However, whether oxLDL regulates mitochondrial fission in PCa and its impact on EMT remains unknown. This study aimed to determine the role of oxLDL in mitochondrial fission associated with EMT in a PCa cell model.

Materials and Methods: DU-145 PCa cells were treated with oxLDL in the presence or absence of MDIVI-1, a mitochondrial fission inhibitor. DRP1 activation (pDRP1 Ser616) and EMT markers (E-cadherin, Vimentin) were evaluated by Western blot. Mitochondrial dynamics and cytoskeletal remodeling were assessed using immunocytochemistry.

Results: OxLDL treatment significantly increased DRP1 phosphorylation and induced a shift in EMT marker expression, characterized by reduced E-cadherin and elevated Vimentin levels in DU-145 cells. These effects were partially prevented by MDIVI-1. Microscopy revealed enhanced mitochondrial fission and cytoskeletal remodeling, including actin filament reorganization, consistent with EMT-associated phenotypic changes.

Discussion: Our findings demonstrate that oxLDL promotes DRP1 activation and mitochondrial fission, contributing to EMT induction in prostate cancer cells. This mechanism may represent a novel link between lipid metabolism and mitochondrial dynamics in tumor progression.

Acknowledgment: Fondecyt 1231911

P96. Impact of heat-shock factor 1 over the replication cycle of Herpes Simplex Virus type 1. Gerardo Ortiz¹ (gqortiz@uc.cl), Rodrigo Reyes¹, Abel Soto¹, Alexis M. Kalergis^{1,2}, Susan M. Bueno¹, Pablo A. González¹

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Introduction: Heat-shock factor 1 (HSF1) is a transcription factor present in most organisms that participates in the maintenance of cellular proteostasis upon exposure to elevated temperatures (39 to 43°C), which can occur because of external heat sources, such as burns, or internal, such as febrile episodes. Additionally, HSF1 has also been shown to have important roles during viral infections regardless of Heat-shock and plays important roles in the transcription of viral genes and viral replication, such as dengue virus (DENV), the human immunodeficiency virus (HIV), human cytomegalovirus (HCMV), and Epstein-Barr virus (EBV), among others. Here, we sought to assess the role of HSF1 over HSV-1 replication in epithelial and neuronal cells

Materials and Methods: We assessed HSF1 activation in HSV-1-infected epithelial cells and neurons (HeLa and SH-SY5Y cell lines, respectively) using luciferase reporters. Additionally, HSV-1 replication was assessed using flow cytometry, fluorescence microscopy, RT-qPCR, and PFU assays in the presence of drugs that negatively and positively modulate HSF1 activation, as well as HSF1 knock-out cells.

Results: We found that HSV-1 activates HSF1 during infection, and that negative modulation of HSF1 activity in HSV-1-infected cells alters the viral replication cycle, resulting in reduced PFU in the supernatants, fewer viral genome copies, and decreased virus-associated GFP reporter fluorescence.

Discussion: Our results suggest that HSF1 plays a relevant role in the replication cycle of HSV-1 in epithelial and neuronal cells. Targeting this host factor may have therapeutic potential as an alternative treatment to reduce the pathological effects of this virus, which remains to be assessed in an *in vivo* model of infection.

Acknowledgment: Millennium Institute on Immunology and Immunotherapy, Facultad de Ciencias Biológicas, Pontificia Universidad Católica, Santiago, Chile. FONDECYT Regular 1240971. We are grateful to Dr. María Estela Andrés for sharing the pHsp70 reporter plasmid.

P97. Prostate Cancer Cells and the Tumor Microenvironment Promote LDL Oxidation. Pampaloni P (ppampaloni2019@udec.cl). Durán A., Azocar S., Castillo M, Duprat F., Olivas G., Rivas Y., Sanzana J., González-Chavarría I.

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Introduction: Prostate cancer (PCa) incidence and progression have been associated with dyslipidemias characterized by elevated low-density lipoprotein (LDL) levels. However, in PCa, native LDL receptor expression is downregulated, while scavenger receptors (SCRs) are upregulated. SCRs do not recognize native LDL but instead bind oxidized LDL (oxLDL). In atherosclerosis, LDL oxidation does not occur in circulation but rather within the arterial intima, where LDL particles are retained by biglycans. This localized environment, enriched in reactive oxygen species (ROS) and inflammation, promotes LDL oxidation and uptake by macrophage SCRs. Similarly, the tumor microenvironment (TME) in cancer consists not only of tumor cells but also of immune and stromal cells embedded in the extracellular matrix (ECM), generating conditions rich in ROS. Moreover, biglycans have been reported to be elevated in various cancers. Our group has shown that oxLDL promotes epithelial-mesenchymal transition (EMT), biochemical progression, and resistance to androgen deprivation therapy (ADT) in PCa. However, the origin of oxLDL in the TME has not been fully elucidated. This study aimed to determine the contribution of the TME to LDL oxidation and to evaluate the presence of native LDL and oxLDL in PCa tissues compared with normal prostate.

Materials and Methods: A transwell system (0.4 µm) was used to assess the role of PCa cells and macrophages (M2, TAM-like) in LDL oxidation. Native LDL was placed in the lower chamber, while PCa cells or macrophages were cultured in the upper chamber. LDL oxidation was evaluated by dot-blot using antibodies against oxLDL or oxidized lipids, normalized to ApoB100. Immunohistochemistry (IHC) was performed to assess ApoB, biglycans, and oxidized phospholipids (OxPLs) in PCa patient tissues, and relative expression was quantified using ImageJ.

Results: A significant increase in LDL oxidation was observed in PCa and PCa/macrophage co-cultures compared to controls after 48 hours. PCa tissues exhibited increased ApoB100, OxPLs, and biglycans compared to normal prostate tissues.

Discussion: These findings demonstrate that PCa cells and TME-like conditions can induce LDL oxidation and that ApoB, biglycans, and OxPLs are elevated in PCa tissues relative to normal prostate.

Acknowledgment: FONDECYT 1231911

P98. Herpes simplex virus type 1 modulates the expression of Fc-Gamma Receptors in dendritic cells. Ignacio A. Pastén-Ferrada (ipastenf@uc.cl)¹, Felipe A. Cancino¹, Areli J. Navarro¹, Abel A. Soto¹, Susan M. Bueno¹, Alexis M. Kalergis^{1,2}, Pablo A. González¹.

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Introduction: Herpes simplex virus type 1 (HSV-1) is a highly prevalent virus that persistently infects humans and elicits numerous diseases. Importantly, HSV-1 infects dendritic cells (DCs), which are professional antigen-presenting cells that initiate and regulate antiviral immune responses. Notably, HSV-1 impairs DC maturation and migration, negatively modulates their T cell-activating capacity and elicits their death. Fc-gamma receptors (FcγRs) expressed on the surface of DCs mediate antigen-antibody immune-complex capture and antigen processing for antigen presentation to T cells via MHC-I and MHC-II. Whether HSV-1 modulates FcγR expression and their function in DCs remains unclear. The aim of this work is to characterize HSV-1-induced changes in FcγR expression in DCs comparing transcriptomic, proteomic and surface-expression readouts.

Materials and Methods: Bone-marrow-derived murine DCs were infected with HSV-1 and analyzed at defined times post-infection. Global transcriptional changes were assessed by RNA-seq and qPCR; protein abundance was determined by mass-spectrometry proteomics; and surface receptor expression was measured by flow cytometry. Pathway analyses focused on FcγR signaling and FcγR phagocytosis.

Results: RNA-seq revealed downregulation of FcγR-mediated phagocytosis pathways at 12 hours post-infection, while qPCR showed divergent FcγR transcript changes: increased FcγRI and FcγRIV mRNA levels but decreased FcγRIII mRNA levels. Proteomics indicated no substantial net change in total FcγR protein levels. In contrast, flow cytometry showed increased surface expression of FcγRI (CD64) and FcγRIV (CD16.2), whereas FcγRIIB (CD32B) and FcγRIII (CD16) remained unchanged.

Discussion: HSV-1 elicits a complex regulation of FcγR expression in DCs visible depending on the method used, with discordant transcriptional, total-protein and surface-expression patterns. FcγR expression alterations may reconfigure FcγR-dependent antigen uptake and signaling, potentially affecting antigen presentation and antiviral T cell responses. Further functional studies are needed to determine consequences regarding antiviral DC-mediated immunity.

Acknowledgment: Millennium Science Initiative Program – ICN2021_045: Millennium Institute on Immunology and Immunotherapy. FONDECYT grant #1240971 from ANID.

P99. FchDOF1 regulates color-associated genes in *Fragaria chiloensis* via the phenylpropanoid/anthocyanin pathway. Nicole Pizarro-Vásquez (npizarro@alumnos.utalca.cl), Raúl Herrera, María Alejandra Moya-León. Functional Genomics, Biochemistry and Plant Physiology Group, Instituto de Ciencias Biológicas, Universidad de Talca.

Introduction: Fruit color is a key trait for strawberry quality and commercial value. In *Fragaria chiloensis* fruit color is determined by anthocyanins synthesized through the phenylpropanoid/anthocyanin pathway. Although several transcription factors have been linked to the regulation of this biosynthetic route, the specific role of FchDOF1 remains uncharacterized. This study aims to evaluate the functional role of FchDOF1 in regulating the expression of anthocyanin biosynthetic genes and its impact on fruit color. For this purpose, the transient overexpression or repression of *FchDOF1* in *F. chiloensis* fruit was performed and the effect was tested by gene expression (qPCR) and phenotypic analyses.

Materials and Methods: Developing *F. chiloensis* fruits (stage 2) were agro-infiltrated with *FchDOF1* overexpression construct (OE fruit), RNAi construct targeting *FchDOF1* (RNAi fruit), or empty vectors as controls. Subsequently, the expression of key genes of anthocyanin/proanthocyanidin pathway was evaluated by qPCR, and fruit color was measured (a* color).

Results: The overexpression of *FchDOF1* rises the expression of *CHS*, *CHI*, *F3H*, *DFR1* and *UFGT* that directly participate in anthocyanins biosynthesis, and represses *LAR* involved in flavonols biosynthesis. This

transcriptomic change is concordant with the advancement in color from green to red observed in OE fruits. In *FchDOF1* silenced fruit the repression of *CHI*, *DFR1* and *ANS* was confirmed in agreement with the absence of color changes. Finally, RNAi fruits also experimented the repression in *ANR* and *LAR* involved in proanthocyanidin biosynthesis.

Discussion: These results suggest that FchDOF1 participates in the regulation of color development in *F. chiloensis* fruit. The overexpression of *FchDOF1* promotes the expression of key genes driven to anthocyanin biosynthesis that coincides with fruit color change (green to red) associated to ripening. In contrast, the silencing of *FchDOF1* reduces the expression of key color associated genes, and the fruit maintains its initial color.

Acknowledgment: FONDECYT 1210948 and 1251538.

P100. Biochemical and Structural Characterization of Oligomeric States of Phosphoenolpyruvate Synthase from the mesophilic archaeon *Methanococcus maripaludis*. Gonzalo Quiñones-Pérez (gonzalo.quinones.p@ug.uchile.cl), Sebastián M. Muñoz, Leslie Hernández-Cabello, Víctor Castro-Fernández, Felipe González-Ordenes and Victoria Guixé.

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Introduction: Phosphoenolpyruvate synthase (PEPS) catalyzes the first step of gluconeogenesis, converting pyruvate and ATP into phosphoenolpyruvate, AMP, and Pi. In bacteria, PEPS exists in a monomer–dimer equilibrium, whereas in thermophilic archaea it assembles into large homo-oligomeric complexes of up to 24 subunits (≈ 2.25 mDa). The factors governing this oligomeric equilibrium in archaea, and the enzymatically active states, remain unclear.

Materials and Methods: To investigate these questions and to determine whether high-molecular-weight oligomers also occur in mesophilic archaea, we characterized the enzyme from *Methanococcus maripaludis* (MmPEPS), providing the first biochemical and structural analysis of a mesophilic archaeal PEPS.

Results: SEC-MALS data revealed that MmPEPS exists in a homo-oligomeric equilibrium similar to that reported for enzymes from thermophilic *Archaea*, forming monomers, dimers, and a 16-subunit multimer (MmPEPS_o). Negative-stain electron microscopy showed a monodisperse, globular structure with an approximate diameter of 16 nm and non-preferred orientation. Preliminary HPLC-SEC results indicate that ATP and the reducing agent DTT promote the dissociation of MmPEPS_o, shifting the equilibrium toward the dimeric state, a phenomenon not observed in the presence of AMP and pyruvate. Finally, enzymatic assays revealed that MmPEPS_o had a higher specific activity (2.3×10^{-3} U/mg) than the dimeric state (8.3×10^{-4} U/mg).

Discussion: Together, these findings indicate that PEPS from *M. maripaludis* exist in a dynamic equilibrium between dimers and a 16-subunit multimer, with both forms being catalytically active. This study establishes a framework for understanding how oligomerization contributes to the regulation of gluconeogenesis in mesophilic archaea.

Acknowledgment: FONDECYT Regular N°1231263.

P101. Herpes simplex virus type 1 impacts glycolytic metabolism and mitochondrial respiration at early stages of infection in dendritic cells. Francisco Romero^{1,2} (faromero3@uc.cl), Andrea Matamoros³, Claudia A. Riedel^{1,4}, Susan M. Bueno^{1,2}, Álvaro Elorza^{1,3}, Pablo A. González^{1,2}.

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Introduction: Dendritic cells (DC) are key immune cells that initiate and regulate host antiviral immune responses. Notably, these cells experience metabolic changes, such as glycolysis upregulation and reduction of mitochondrial respiration when they interact with pathogens or their related components and initiate a maturation process. Herpes simplex virus 1 (HSV-1) is a pathogen that is highly prevalent in the human population and can infect epithelial cells, neurons, and immune cells, including DCs. During infection, HSV-1 inhibits the immune

functions of DCs, reducing the expression of co-stimulating molecules that support the activation of T cells and antigen presentation. This work aims at determining the metabolic profile of murine DCs at early stages upon HSV-1 infection.

Materials and Methods:

Bone marrow-derived dendritic cells (BMDCs) were assessed at 3, 6, 9, and 12 h after infection. We used untreated cells and mock-inoculated cells as controls at the same time points. Real-time metabolic analysis was performed using a Seahorse XF96 setting to evaluate the extracellular acidification rate (ECAR) and the oxygen consumption rate (OCR), with both parameters directly associated with glycolysis and mitochondrial respiration, respectively.

Results: We observed a significant upregulation of ECAR values at 9 and 12 h post-infection with HSV-1. Furthermore, basal glycolysis and glycolytic capacity were significantly upregulated during infection. OCR also displayed significant difference at 12 h post-infection; basal respiration as upregulated, but the maximal respiratory capacity was altered, showing a decrease when compared to the untreated and mock controls. These results suggest that the reserve capacity of DCs is significantly reduced upon infection at 12 h post-virus inoculation.

Discussion: Taken together, the results suggest that HSV-1 modulates DC metabolism upon infection, altering glycolysis and mitochondrial respiration, which could be related to impaired function of these cells.

Acknowledgments: Millennium Institute on Immunology and Immunotherapy #ICN2021_045, FONDECYT Regular 1240971, FONDECYT Regular 1250762. Fondecuip EQM220115

P102. Allulose acutely activates arcuate NPY neurons and differentially affects intake and body weight.

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Introduction: The hypothalamus regulates energy balance via the arcuate nucleus (ARC), which contains orexigenic NPY/AgRP and anorexigenic POMC/CART neurons. Excess intake of simple sugars such as sucrose contributes to metabolic disease. Allulose is a low-calorie rare sugar with potential metabolic benefits, but its central effects are unclear. We examined whether acute allulose exposure modulates ARC neuronal activity.

Materials and Methods: Transgenic POMC-GFP and NPY-GFP mice (11–14 weeks old) were assigned to allulose, sucrose, or water for 7 days. Food intake, solution consumption, and body weight were recorded. 45 minutes after exposure, brains were processed for fluorescent immunohistochemistry, and neuronal activation was quantified by c-Fos expression in ARC NPY and POMC neurons. We also assessed glial responses (GFAP for astrocytes).

Results: Acute allulose dramatically increased c-Fos in ARC NPY neurons compared with water and sucrose, while POMC neurons showed no significant change. Under identical conditions, sucrose did not increase ARC c-Fos relative to water. After 7 days of sucrose ingestion, astrocytic activation (GFAP) was unchanged. Food intake and solution consumption markedly changed depending on the sugar consumption. We evidenced an acute weight loss in males but not females on allulose group.

Discussion: These findings indicate that allulose, despite its negligible caloric content, rapidly engages orexigenic ARC circuits, whereas sucrose does not under the acute conditions tested. Together, the data support a model in which allulose modulates hypothalamic activity differently from sucrose and may influence feeding behavior and energy balance via NPY neurons.

P103. SURF4, a New Regulator of TRPC6 Ion Channel Activity. Evrin Servili (servili.evrin@gmail.com),

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Introduction: Transient Receptor Potential Canonical 6 (TRPC6) is a nonselective cation channel implicated in various physiological and pathological conditions, including cancer progression. TRP channels regulate membrane potential through ion transport, and their proper trafficking is essential for their function. We aimed to discover new binding partners of the TRPC6 channel by performing affinity immunoprecipitation and proteomics experiments. This led to the discovery of SURF4, a cargo receptor that mediates ER-to-Golgi transport. Our work explores how SURF4 influences TRPC6 trafficking and activity.

Materials and Methods: We performed affinity immunoprecipitation and spectrometry-based proteomics experiments to identify the TRPC6 interactome in HEK293 cells. The interaction was validated using immunoprecipitation assays and Bioluminescence Resonance Energy Transfer (BRET) assays in HEK293 cells. To study the effect of SURF4 on TRPC6 trafficking, we used RUSH assay and cell surface protein biotinylation assays to assess its localization. To measure the effect on channel activity, we conducted intracellular calcium imaging experiments using the Fura-2 AM probe stimulating cells with the specific TRPC6 activator, Hyperforin in the presence or absence of 4-PBA, a SURF4 inhibitor.

Results: Our findings confirm that SURF4 is a novel binding partner for TRPC6. This interaction actively facilitates the export of TRPC6 from the ER to the Golgi apparatus, as evidenced by an increased Pearson's colocalization score between TRPC6 and the Golgi marker when SURF4 is overexpressed. Calcium imaging experiments showed that SURF4 overexpression leads to a reduction in Hyperforin-induced calcium influx.

Discussion: This study identifies SURF4 as a new regulator of TRPC6 activity. SURF4 facilitates the trafficking of the TRPC6 channel from the ER to the Golgi. The decrease in channel activity despite increased membrane presence suggests that SURF4 negatively modulates the function of TRPC6.

Acknowledgment: Fondecyt #1240633 (OC), #3210419 (BR), #3240187 (ES).

P104. Role of pseudouridine synthase 7 over the replication cycle of HSV-1 in a neuronal cell line. Abel Soto-Machuca^{1,2} (aasoto7@uc.cl), Gerardo Ortiz^{1,2}, Rodrigo Reyes-Ramirez^{1,2}, José P. Rubio^{1,2}, Cecilia Rojas-Fuentes^{1,3}, Aracelly Gaete-Argel^{1,3,4}, Sebastián Giraldo^{1,3,4}, Ricardo Soto-Rifo^{1,3,4} and Pablo A. González^{1,2}.

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Abstract: Herpes simplex virus (HSV-1) replication within host cells requires viral protein expression, which is tightly regulated in infected cells. One mechanism of regulation of viral protein synthesis has been reported to be the chemical modifications of nucleotides within mRNA. Among these modifications is the isomerization of uridine to pseudouridine, which is known to regulate splicing and mRNA translation. This modification is catalyzed by a subset of enzymes called pseudouridine synthases (PUS), where PUS7 catalyzes pseudouridilation in mRNAs. The main aim of this work is to evaluate if PUS7 has a role in the replication cycle of HSV-1 in a neuronal cell line.

Methodology: We used knockdown (KD) of PUS7 expression in the SH-SY5Y neuronal cell line infected with HSV-1 K26-GFP at an MOI of 3, and samples were collected at different times post-infection for flow cytometry analysis, titration of plaque forming units and virus-related reporter expression in infected cells and supernatants, as well as total RNA extraction for RT-qPCR analysis.

Results: A significant decrease in PFUs, and fluorescence MFI of VP26-GFP protein was observed in the PUS7 KD cell line in supernatants and pellets of infected cells after 18 hpi. At early timepoints of infection, a similar amount of PFUs was observed in the scramble and the PUS7 KD cell line. Moreover, while a similar percentage of infected cells was observed at all times post-infection analyzed, a significant decrease in viral mRNA of *icp27* and early gene *tk* and late gene *gB* was observed at 8 hours post-infection.

Discussion: These results suggest that PUS7 is essential for the replication cycle of HSV-1 most probably affecting the biogenesis of the viral mRNAs *icp27*, *tk*, and *gB*.

Acknowledgments: Authors are supported by Fondecyt Regular 1240971 and the Millennium Institute on Immunology and Immunotherapy #ICN2021_045. ASM is supported by ANID PhD fellowship #21241472.

P105. Molecular modeling of the lipopolysaccharide transport (Lpt) system in *Klebsiella pneumoniae*. Constanza Valdebenito^{1,2} (cvaldebenito21@alumnos.utalca.cl), Ariela Vergara-Jaque².

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Introduction: *Klebsiella pneumoniae* is a Gram-negative bacterium belonging to the Enterobacteriaceae family and is a major cause of nosocomial infections. Its outer membrane contains lipopolysaccharides (LPS), essential components that contribute to structural integrity, virulence, and immune evasion. LPS translocation across the cell envelope is mediated by the lipopolysaccharide transport (Lpt) system, an ATP-binding cassette (ABC) transporter composed of seven proteins (LptA–G), which has been proposed as a potential target for disrupting LPS transport. These proteins form two subcomplexes: LptB₂CFG in the inner membrane and LptDE in the outer membrane, linked by the periplasmic protein LptA. Although the overall architecture of the system has been described, the precise assembly dynamics and molecular interactions among its components remain unclear. Therefore, the aim of this work was to employ molecular modeling of the Lpt system in *K. pneumoniae* to better understand its structural organization.

Materials and Methods: Lpt protein sequences from *K. pneumoniae* were obtained from UniProt. Structural models were generated with I-TASSER and AlphaFold, complemented by available PDB templates. Models were visualized and analyzed with PyMOL. Evolutionary coupling analyses were also performed to explore residue-residue interactions.

Results: As a result, structural models of Lpt proteins were generated and protein-protein interactions (LptA–LptA, LptA–LptC, LptA–LptD, LptB₂FGC) were modeled with AlphaFold-Multimer. A complete Lpt assembly was built and analyzed with PyMOL and MolProbity. Evolutionary coupling analyses supported the predicted interfaces and structural consistency.

Discussion: The structural models generated provide a comprehensive view of the Lpt system in *K. pneumoniae*. AlphaFold-Multimer predictions and evolutionary coupling analyses highlight conserved interfaces consistent with experimental data, supporting the reliability of the assembled model. This framework offers a foundation for future functional studies and the identification of therapeutic targets.

Acknowledgment: Proyecto Exploración #13250060

P106. Characterization of transcription factors and their role in drought response in *Eucalyptus globulus*.

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Introduction: *Eucalyptus globulus*, an important forest species for Chile industry, but is highly vulnerable to water scarcity, a growing challenge due to climate change. Plants respond to water stress by modulating gene expression, a process regulated by transcription factors (TFs). This study aims to characterize the role of the *NAC*, *WRKY*, *MYB*, and *AP2/ERF* gene families and their relationship in modulating lignin synthesis.

Materials and Methods: Genes from the TF families were identified in the *E. globulus* genome. Their physicochemical and genetic properties were analyzed. The differential expression of these genes was evaluated in leaf and stem tissues of drought-tolerant and susceptible genotypes. Additionally, the interaction network among these TFs was analyzed.

Results: We identified 189 *NAC*, 87 *WRKY*, 322 *MYB*, and 200 *AP2/ERF* genes, these were grouped into 5, 3, 4, and 4 described subfamilies, respectively. In addition, key genes in lignin biosynthesis were found, such as 8 *PAL*, 25 *CCoAOMT*, and 87 *COMT* genes. Coordinated expression profiles were identified between genes of the lignin biosynthesis pathway and different TFs under water stress. The interaction network revealed key connections between transcription factors and genes of the lignin pathway.

Discussion: This work represents the first large-scale identification of TF families in *E. globulus* and their relationship to drought response. The differential expression of lignin genes regulated by TFs highlights a key plant response mechanism. The results indicate that these TFs not only influence lignin biosynthesis but may also regulate osmotic genes, thus improving drought tolerance.

Acknowledgments: This work was supported by ANID-Anillo project ATE220043 and ANID-Fondecyt project 1241579.

P107. Effect of the overexpression of the Transcription Factor TRIATDRAFT_315146 of *Trichoderma atroviride* in *Solanum lycopersicum* growth and biocontrol of *Botrytis cinerea*. Karla Vargas-Menares^{1,2,3} (k.vargasmenares@uandresbello.edu), Isidora Venegas^{1,3}, Paulo Canessa^{2,3} and Consuelo Olivares-Yáñez^{1,3}.

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Introduction: Gray mold disease, caused by the fungus *Botrytis cinerea*, constitutes a significant threat to global crop production, leading to substantial economic losses. The emergence of fungicide-resistant strains underscores the urgent need for alternative control strategies. This study explores the use of *Trichoderma atroviride* as a biocontrol agent. Previous research by our laboratory has identified transcription factors (TFs) potentially involved in the interaction of *T. atroviride* and phytopathogens. This research focuses on the TF TRIATDRAFT_315146, who based on a gene regulatory analysis, potentially control the expression of peptidases and secondary metabolites. The objective is to evaluate the impact of the TRIATDRAFT_315146 overexpressing strain on tomato plant growth and its efficacy in inhibiting *B. cinerea* growth.

Materials and Methods: An overexpressing *T. atroviride* strain was used to assess its biocontrol activity against *B. cinerea* in dual-culture confrontation assays and inhibition assays. There were photos taken 4, 7, 10, and 14 days post-inoculation, and the percentage of pathogen inhibition was calculated. To evaluate plant growth promotion, tomato plants were inoculated with the overexpressing strains, and fresh and dry biomass were measured fourteen days post-inoculation.

Results: An overexpressing strain was successfully obtained, and its high expression was confirmed by Real-Time PCR. We expect that this strain will show an enhanced inhibitory effect on *B. cinerea* growth. Additionally, we anticipate an increase in fresh and dry biomass in tomato plants inoculated with the overexpressing strain.

Discussion: The expected results may provide evidence that the overexpression of the TF enhances the capacity of the fungus to inhibit the pathogen and promote plant growth.

Acknowledgment: FONDECYT Iniciación 11240968, FONDECYT Regular 1240742, iBio ICN17_022 ICM-ANID

P108. Identification of Key Genes Associated with Biocontrol Potential in *Trichoderma atroviride*. Isidora Venegas^{1,3} (isi.venegas.garrido@gmail.com), Nicolas Arias^{3,4}, José David Fernández^{1,2,3}, Elena Vidal^{1,2,3}, Paulo Canessa^{3,4}, Consuelo Olivares-Yáñez^{1,2,3}.

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Introduction: *Trichoderma atroviride* is a mycoparasitic fungus widely recognized for its role in the biological control of phytopathogens such as *Botrytis cinerea*. Although the stages of mycoparasitism have been described, the transcriptional programs that enable biocontrol remain insufficiently understood. This study aims to explore how gene expression patterns in *T. atroviride* are modulated during early and late stages of interaction with *B. cinerea*.

Materials and Methods: Previously generated RNA-seq datasets from dual-culture assays between *T. atroviride* and *B. cinerea* over a seven-day period under different culture media were analyzed. Transcriptomic analyses and gene regulatory network (GRN) inference were performed to identify key candidate genes and transcription factors involved in regulatory transitions. Additionally, *T. atroviride* overexpressing strains were generated for functional validation of candidate genes via *in vitro* and *in vivo* confrontation assays, aimed at evaluating their potential to enhance efficacy against *B. cinerea*.

Results: Preliminary analyses revealed the identification of transcription factors and key genes potentially involved in the regulation of *T. atroviride* biocontrol activity. Current progress includes the generation of homokaryotic overexpressor lines for a subset of candidate genes. Functional evaluation of these strains in confrontation assays with *B. cinerea* is ongoing to determine their potential to enhance biocontrol, although further experimental validation is still required.

Discussion: These findings provide a foundation for understanding the regulatory mechanisms underlying *T. atroviride* mycoparasitism. The identification of candidate genes through RNA-seq and GRN analyses and the generation of overexpressing strains establish a framework to uncover genes that modulate biocontrol activity. Functional evaluation of these candidates through *in vitro* confrontation assays and future *in planta* tests will determine their potential to enhance the growth inhibition of *B. cinerea*.

Acknowledgment: FONDECYT Iniciación 11240968, FONDECYT Regular 1240742 y 1250631, iBio ICN17_022 ICM-ANID

P109. Discovery and characterization of compounds that disrupt MYC/NSD3S oncogenic interaction. Sebastián Vera¹ (savera@ucsc.cl), Diego Acosta Yáñez², Matías Hepp^{1,4}, Carlos Farkas^{1,4}, Alexis Salas-Burgos^{2,4}, Yuhong Du³, and Valentina González-Pecchi^{1,4}.

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Introduction: The MYC oncogene, known for its crucial role in the regulation of cell proliferation in multiple cancer types, has been the subject of study for the development of new therapeutic agents for many years. However, still there is no MYC inhibitor approved by the FDA. We described a new oncogenic interaction between MYC and NSD3S. In this project, our aim was to identify and characterize compounds that were able to disrupt the MYC/NSD3S oncogenic interaction.

Material & Methods: We used ultra-high throughput screening (uHTS) assays on 1536-well plates to test different compounds from the MedChemExpress (MCE) library. The live cell NanoLuc-based Protein-Fragment Complementation Assay (NanoPCA) was used as the primary screen to evaluate the interaction between MYC/NSD3S with or without compounds. To validate the positive hits, we employed complementary biophysical and computational approaches; Thermal Shift Assay (TSA), Surface Plasmon Resonance (SPR), and *in silico* molecular dynamics (MD) simulations. Finally, immunoprecipitation assays were used as a secondary method to confirm the disruption of the MYC/NSD3S interaction.

Results: We screened over 12,000 compounds and the percentage hit rate of the screening assay was 0.1%, with 12 compounds capable of modulating the MYC/NSD3S interaction and decreasing MYC transcriptional activity as a secondary readout. The validation of these positive compounds was carried out using the previously described methodologies. Of all the candidates, two compounds, ethoxysanguinarine and Withaferin A, stood out for showing an optimal effect on the MYC/NSD3S interaction, confirming their significant potential as modulators.

Discussion: Our discoveries highlight a list of promising compounds for disrupting the MYC/NSD3S interaction. By integrating NanoPCA with complementary biophysical and computational techniques, we have developed a powerful platform for designing targeted therapies against MYC-mediated interactions, taking steps in the direction of developing a therapy for MYC-driven tumors.

Acknowledgments: PAI77200098 (VGP), FAA2025 (VGP), Proyecto USC 20102 (SV)

P110. Implementation of a tool based on Surface Plasmon Resonance for the functional characterization of the p.Asp47Asn variant of the LDL receptor associated with familial hypercholesterolemia. Noemí Vilches¹ (novilches2019@udec.cl), Maximiliano Figueroa², Andrea Sánchez¹.

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Introduction: Familial Hypercholesterolemia (FH) is a prevalent genetic disorder associated with high morbidity and mortality due to early cardiovascular disease. The main cause is mutations in the LDL receptor gene (LDLR); one of the most frequent in Chile is p.Asp47Asn, classified as a variant of uncertain significance (VUS). Prior clinical and *in silico* studies suggest its pathogenicity, with carriers showing elevated LDL-C levels and computational models indicating reduced ApoB100 binding affinity. Experimental affinity characterization is required to confirm this. Surface Plasmon Resonance (SPR) emerges as a powerful technique, capable of assessing affinity variations and evaluating multiple samples in series once standardized.

Materials and Methods: Recombinant ectodomains of wild-type (WT) and p.Asp47Asn LDLR were engineered with an N-terminal erythropoietin secretion signal and a C-terminal 6XHis-Tag. These vectors were expressed in HEK293 cells, and the secreted proteins were purified from the culture medium using IMAC, ion-exchange, and size-exclusion chromatography. Protein identity and purity were verified by Western blot and SDS-PAGE. To determine the dissociation constant (Kd) for LDLR-LDL interaction, a P4PRO+ SPR system with an amine-coupled sensor chip will be used.

Results: The EPO sequence enabled recovery of recombinant proteins from the culture medium. Despite partial loss of the 6X-His tag, purification was achieved through a combination of chromatographic techniques. SPR determined the dissociation constant (Kd) for both WT and p.Asp47Asn LDLR ectodomains in interaction with ApoB100.

Discussion: This work highlights the potential of SPR for functional evaluation of VUS. Our findings are expected to establish the pathogenicity of p.Asp47Asn, contributing to improved genetic diagnosis and patient management in FH.

P111. Identification and Characterization of an Antisense Protein Encoded by Hepatitis B Virus. Cristian Vizueta^{1,2,3} (c.vizuetam@gmail.com), Micaela M Ricca^{1,2,3}, Sebastián Valenzuela^{1,2,3}, Iván Alfaro⁴, Alejandra Loyola^{1,2,3}, & Rodrigo A Villanueva^{2,3}.

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Introduction: Hepatitis B virus (HBV) is a partially double-stranded DNA virus with four canonical reading frames such as P, S, Core, and X, and all encoded on the negative strand of its genome.

Materials and Methods: Consistent with previous reports, we identified a conserved reading frame on the positive strand overlapping the HBx region in the opposite orientation, suggesting the presence of an antisense protein, provisionally named **HBy**. Sequence analysis revealed that HBy is highly conserved across HBV genotypes, encoding a 95-amino acid protein (≈ 10.5 kDa; pI 11).

Results: Bioinformatic analysis predicted several post-translational modification sites, and a 3D structural model was generated. Recombinant HBy was expressed in *E. coli*, purified for preliminary biochemical characterization, and used to produce polyclonal antibodies. HBy was also ectopically expressed in mammalian cells to determine its subcellular localization by immunofluorescence and to evaluate its impact on secretory markers of viral replication in hepatocellular carcinoma cells transfected with HBV genomes.

Discussion: These findings reveal a novel antisense-encoded HBV protein, expanding the known coding potential of HBV, suggesting a possible role in viral pathogenesis and as a potential antiviral target.

Acknowledgment: Fondecyt Regular 1240409, Centro Ciencia & Vida, FB210008, Financiamiento Basal para Centros Científicos y Tecnológicos de Excelencia.

