XLVII Annual Meeting

Chilean Society for Biochemistry and Molecular Biology

2024

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CONFERENCES ABSTRACT

IUBMB OPENING LECTURE

Programming of Mitochondria by Proteolysis.
Thomas Langer (tlanger@age.mpg.de), Max-Planck-Institute for Biology of Ageing, Cologne, Germany.

Mitochondria are essential metabolic organelles and integral part of numerous cellular signaling pathways. Cellular signals determine the composition of the mitochondrial proteome and the metabolic output of mitochondria, which influence cell fate during development, cell differentiation, in ageing and disease. Mitochondrial proteases are emerging as central regulators of these adaptive responses. The i-AAA protease YME1L regulates in concert with the stress-activated peptidase OMA1 mitochondrial fusion via OPA1 and couples mitochondrial shape and metabolic function. Impaired pyrimidine synthesis in the absence of YME1L causes the release of mtDNA to the cytosol and cGAS-STING dependent inflammation. YME1L activation promotes growth of pancreatic ductal adenocarcinoma cells and preserves the self-renewal capacity of adult neural stem cells. OMA1 also promotes mitochondrial stress signalling in OXPHOS-deficient cardiomyopathy and protects against ferroptosis. Mitochondrial proteases thus coordinate mitochondrial dynamics and metabolic functions at the interface of inflammation and cell death.

PABMB PLENARY CONFERENCE

Integrins: Environment recognition machines activated by ligand binding and actin pulling

Dr. Timothy Springer, Harvard Medical School, USA.

All animals have integrins, heterodimeric cell surface adhesion receptors that link the extracellular environment to the cytoskeleton. Among multiple families of adhesion molecules, only integrins mediate cell migration. Mammals have 18 α and 8 β-subunits that form 24 integrins that recognize diverse ligands on cell surfaces and in extracellular matrix. Twelve extracellular domains have been conserved in integrin α and β-subunit architecture for at least 600 million years in metazoans. In machine-like integrin conformational movements, these moving parts rearrange to increase affinity ~2,000-fold in the 22 "typical" integrins that have three conformational states and connect to the actin cytoskeleton through adaptors such as talin.
Rapid coordination between events outside and inside the cell is required for integrins to mediate cell spreading and migration. Single-molecule fluorescence dynamics show that ligand binding to the bent-closed integrin conformation, which predominates on cell surfaces, is followed within milliseconds by two concerted changes, leg extension and headpiece opening, to give the high-affinity integrin conformation. The extended-closed integrin conformation is not an intermediate but can be directly accessed from the extended-open conformation and provides a pathway for ligand dissociation. In contrast to ligand, an adaptor that links the integrin beta-subunit cytoplasmic domain to the actin cytoskeleton modestly stabilizes, but does not induce, extension or opening. Integrin activation is thus initiated by outside-in signaling and followed by inside-out signaling. Our results further imply that adaptor binding is insufficient for inside-out integrin activation and that tensile force transmission through the ligand-integrin-adaptor-actin cytoskeleton complex is required.

Lin, F. Y., Li, J., Xie, Y., Zhu, J., Huong Nguyen, T. T., Zhang, Y., Zhu, J., and Springer, T. A. (2022) A general chemical principle for creating closure-stabilizing integrin inhibitors. Cell 185, 3533-3550 e3527, PMID: 36113427

OSVALDO CORI CONFERENCE

Hemocyanins of mollusks, giant microscopy used in biomedicine and biotechnology: Exploring their structure with relation to their mechanisms of action on the immune system of mammals

María Inés Becker, Biosonda S.A., y Fundación Ciencia y Tecnología para el Desarrollo (FUCITED)

The respiratory glycoproteins, hemocyanins, have numerous applications, such as nonspecific carrier protein-based adjuvants for both human and veterinary purposes. They are immunogenic but do not cause adverse effects, which makes them safe for use as immunomodulators. For over 40 years, Megathura crenulata hemocyanin, also known as Keyhole limpet hemocyanin (KLH), has been the standard for these applications. It was unknown whether other hemocyanins had similar immune effects until our laboratory found that Concholepas concholepas hemocyanin (CCH) has comparable immunostimulatory properties.

Our research goals have been to understand the structural elements of CCH and KLH, which differ in their quaternary subunit organization, and to elucidate the underlying mechanisms of their immunostimulant properties in mammals. Hemocyanins have unique mannose-rich N-glycan oligosaccharides that make them multiligands, i.e., they interact with several innate immune receptors on the antigen-presenting cells (APCs). These receptors belong to the C-type lectin-like family, including the mouse and human mannose receptor (MR), human DC-SIGN, and Toll-like receptors like TLR4. The simultaneous binding of hemocyanins to different innate immune receptors, particularly to TLR4, which has unique dual signaling capabilities, induces distinct signaling pathways, providing the diversity required to shape an effective adaptive immune response. Moreover, since hemocyanins possess a high molecular mass (8 MDa), they are slowly processed as exogenous antigens, generating peptides loaded onto the major histocompatibility complex II (MHC-II). Surprisingly, hemocyanins are also processed through cross-presentation in the MHC-I pathway. Overall, hemocyanins promote a strong bias towards type 1 T helper (Th1) polarization, creating a valuable immunological bystander effect.

FEBS WORLDWIDE PLENARY LECTURE

Oncogenes manipulate innate immunity during injury, repair, tumorigenesis and tumor regression.

Gerard Evan, The Francis Crick Institute and Kings College London, London, UK.

Of late, it has become clear that many adult cancers exhibit some degree of local immune suppression and evasion of surveillance by the immune system. Such immune evasion is usually depicted as a consequence of the strong initial selection against tumors and their neo-antigens: however, our data indicate that suppression of both adaptive and innate immunity are inherent properties of mitotic oncogene activity in both pathological (neoplasia) and physiological (reparative) tissue growth and regeneration. For example, in mouse models of both lung and pancreatic adenocarcinoma, as well as a liver injury model, acute activation of Myc triggers rapid influx of inflammatory monocytic and granulocytic cells along concurrently with immediate exclusion of lymphocytes (abT, B and NK). Moreover, many of these Myc-driven immune manipulations are essential for the transition of indolent pre-tumors into malignant neoplasms. Similarly, CCl4 liver injury triggers an immediate, abrupt and Myc dependent exclusion of lymphoid cells and influx of monocytic and granulocytic that is essential for successful liver repair. Moreover, in liver the switch from post-injury regeneration to injury resolution is dependent upon Myc down regulation and re-influx of innate immune NK-like cells. Taken together, our data indicate that cancer is an aberrantly “hacked” persistent version of normal post-injury tissue repair and regeneration. Conversely, blocking Myc (or upstream oncogenic signals) is therapeutically efficacious in cancer because forcibly turning Myc off “hacks” the physiological tissue injury resolution program by which innate immune cells prune supernumerary tissue and return the “repaired” tissue to its normal size, architecture and function. "

SEVERO OCHOA LECTURE

Nature´s strategic decisions: dormancy and activation in plant meristems steps.
Pilar Cubas, Eduardo González-Grandío, Michael Nicolas, Aitor Muñoz, Yan Long, Gema Castillo. Centro Nacional de Biotecnología-CSIC, Madrid, Spain

In higher plants, branch development is a key developmental process with significant impact on aboveground architecture. Shoot branching patterns determine leaf, flower and fruit production, and thus reproductive success and agricultural yield. Axillary buds, the branch primordia, emerge in the leaf axils, and their subsequent growth or dormancy is regulated at the plant-wide level. Axillary bud activity is influenced by a complex interplay of environmental and endogenous signals that relay information regarding conditions such as nutrient and water availability, light quality and status of sink/source organs. This information is translated into a local response, in the axillary buds, of growth or quiescence.
Genetic mechanisms underpin the regulation of shoot branching. A key genetic component in this regulatory network is the gene encoding the TCP transcription factor BRANCHED1 (BRC1), which acts within axillary buds to suppress shoot branching. BRC1 acts as a central integrator of endogenous cues and environmental factors that influence bud activity, and triggers global transcriptomic changes that promote bud dormancy. We are elucidating the signaling pathways that converge in BRC1 regulation, and we are also exploring the gene regulatory networks downstream of BRC1, essential for bud growth cessation. In addition, we are studying the conservation and evolution of the BRC1 gene in angiosperms. We have analyzed in detail the evolution of BRC1 in the Solanaceae family, that comprises species of agronomical interest such as potato and tomato, and found novel functions for BRC1 in these species.

SYMPOSIUM ABSTRACTS

Symposium 1: Epigenetic control of cell function

Single cell data to examine molecular signatures in adaptive immune cells in the context of obesity. Dr. Marcela Sjöberg, Pontificia Universidad Católica de Chile, Chile

The immune system maintains a delicate balance between host protection against infections and tolerance of the body's own cells. The immune response is achieved through innate and adaptive systems, which depend on the correct establishment of cell type specific gene expression programs. Crucial aspects of adaptive immune responses rest on CD4+ regulatory T cells (Tregs), such as regulation of immune homeostasis, self- tolerance, inflammatory responses and tissue homeostasis. Studies have shown that Treg cells in lymphoid and non-lymphoid tissues hold unique characteristics. In obesity, a chronic low-grade inflammation, immune aging, and markers of T cell exhaustion are reported, yet a deeper characterization of these subpopulations to elucidate the gene expression programs underlying the imbalance on tissue-resident T cell subpopulations is missing in the context of obesity. To gain insights into the biological basis of immune system dysregulation in obesity and identify pathways altered in populations of Treg cells we are interrogating single-cell RNA sequencing (scRNA-seq) data sets to characterize cellular heterogeneity at a deeper resolution and examine obesity-associated immune T cell phenotypes and gene expression signatures characterizing tissue-resident Treg cells to reveal specific molecular and metabolic signatures on them. We are also exploring underlying epigenetic mechanisms regulating changes in gene expression and working on developing a bioinformatic tool to annotate cell types based on surface protein levels using cellular indexing of transcriptomes and epitopes by sequencing (CITEseq).
Acknowledgment: This work is supported by grants from ANID- FONDECYT REGULAR 1241935 and the Chan Zuckerberg Initiative (CZI).

Epigenetic editing: from criticized research tool to realistic clinical translation in only a decade. Marianne G Rots (m.g.rots@umcg.nl), Dept of Pathology and Medical Biology, University Medical Center Groningen/University of Groningen, the Netherlands.

Epigenetic editing, the controlled writing or erasing of epigenetic signatures on a given genomic locus, is quickly developing. Various animal models demonstrated that such epigenetic reprogramming of gene expression is therapeutically effective, and that effects can be maintained. With at least ten pharmaceutical companies founded to translate epigenetic editing to the clinic, expectations of this technology as new tool in the clinical arena are high. In this presentation, I will give a historical overview from the coining of the term by us[1] and the initial disbelief, to its current status[2] as flexible research tool and a

clinically realistic, less invasive, yet equally potent alternative to (CRISPR-based) gene editing.

[1]De Groote, Verschure, Rots: “Epigenetic Editing: targeted rewriting of epigenetic marks to modulate expression of selected target genes”. Nucl Acids Res 2012
[2]Heller, Bintu, Rots: “Epigenetic editing tools: Advances and Challenges”. Nature Reviews Drug Discovery (in prep)

New strategies to target macrophages in health and disease. Dr. Alejandro Villagra, Georgetown University, USA.

HDAC inhibitors (HDACis) have been primarily used as anticancer drugs due to their ability to impair tumor cell proliferation and survival. However, several research groups, including us, have shown that the intervention of some particular histone deacetylases (HDACs) differentially affects immune-related pathways. These functional characteristics, primarily unexplored and distant from their canonical cytotoxicity-centered role over cancer cells, are expanding the potential use of HDAC inhibitors (HDACis) as attractive targets to modulate immune cells in the context of other conditions and diseases such as immunotherapy, neurodegeneration, autoimmune diseases, fibrosis, and tissue repair among others. Although these non-canonical effects have been extensively reported, the molecular mechanisms are not entirely understood.
Supporting the above premise, we have found that ultra-selective HDAC6is and HDAC10is have promising antitumor effects and can promote a pro-inflammatory tumor microenvironment (TME). Recent results from our group suggest that the *in vivo* antitumor effect of these HDACis is mainly mediated by their regulatory role over the phenotype and functional activity of tumor-associated macrophages (TAMs). TAMs, primarily M2-polarized macrophages, exhibit detrimental effects on antitumor T cell function and are strongly associated with a poor prognosis in cancer. In the opposite direction, we have found that the pharmacological and genetic intervention of HDAC11 promotes the wound-healing macrophage M2 phenotype, mainly by modulation of inflammatory pathways in macrophages.
All the above suggests that HDACis could influence several cellular mechanisms unrelated to their cytotoxic effect on cells. Therefore, developing novel selective HDACis and insights into the molecular and cellular mechanisms involved in these processes are needed to identify novel therapeutic targets to modulate immunomodulatory pathways in the context of cancer and multiple other diseases and conditions.

Epigenetic control during learning and memory. Dr. Martín Montecino, Universidad Andrés Bello, Chile

Control of gene transcription is necessary during the formation and consolidation of contextual hippocampal memories. Fine-tuning of these gene transcription events involves epigenetic post-translational modifications at chromatin-associated histone proteins (HPTMs) together with high-order chromatin rearrangements that allow distal enhancer-mediated control. HPTMs associated with active transcription, including H3K4me3 and H3K27ac, have been found globally increased in the hippocampal CA1 region during contextual fear conditioning (CFC), a strong Pavlovian learning paradigm. However, their role at neuroplasticity-related promoters in neurons from mice subjected CFC training have not been established. We have obtained a detailed epigenetic profile of fear-activated hippocampal neuron populations *in vivo* that can subsequently allow evidencing epigenetic mechanisms regulating gene transcription during hippocampal memory consolidation. We measured early memory formation at 1h-post-CFC and early-memory consolidation at 24h-post-CFC. RNA-seq analyses of the CA1 region revealed gene clusters reflecting different transcriptional profiles occurring at 1h-post-CFC and 24h-post-CFC. ChIP-seq analyses indicated that a number of these genes exhibit H3K4me3 and H3K27ac enrichment on their promoters along with an increase in their mRNA expression in hippocampal CA1 neurons. Finally, Hi-C analyses revealed that many of these gene promoters are regulated in cis by distally located putative enhancer elements. Together, our results support a model where epigenetic mechanisms and high-order chromatin organization contribute to the fine-tuning of transcription during early fear memory formation.
Acknowledgment: Work funded by ANID-MILENIO NCN2023_32, ANID-FONDECYT 1221745 and 1211026.

Symposium 2: New Frontiers in Molecular Mechanisms in Chronic Diseases

Heart disease in the HIV-infected population. Eliseo Eugenin (eleugeni@utmb.edu). Department of Neurobiology, John Sealy School of Medicine, The University of Texas Medical Branch (UTMB), Galveston, Texas, USA.

In this current antiretroviral therapy (ART) era, HIV-associated cardiovascular disease is still significantly higher in people living with HIV despite adherence to therapy. Latently infected cells secrete low levels of viral proteins like HIV-Tat irrespective of ART status, which promotes chronic inflammation. Elucidating novel mechanisms behind HIV/HIV-Tat-induced cardiac damage is critical to understanding HIV-associated cardiovascular disease. Here, we investigate the role of viral reservoirs and the chronic synthesis of HIV-Tat to gap junction/hemichannel communication and their contribution to bystander damage. We demonstrated that HIV and drug use hijack" the cell-to-cell communication systems of the

heart to amplify damage even in the current ART era. HIV-associated neurological dysfunction is observed in more than half of the HIV-infected population, even in the current antiretroviral era. The mechanisms by which HIV mediates CNS dysfunction are not well understood but have been associated with the presence of long-lasting HIV reservoirs. In the CNS, macrophage/microglia and a small population of astrocytes harbor the virus. However, the low number of HIV-infected cells does not correlate with the high degree of damage, suggesting that mechanisms of damage amplification may be involved. Here, we demonstrate that the survival mechanism of HIV-infected cells and the apoptosis of surrounding uninfected cells is regulated by inter-organelle interactions among the mitochondria/Golgi/endoplasmic reticulum system and the associated signaling mediated by IP3 and calcium. We identified latently HIV-infected cells protected from apoptosis but compromised neighboring cells by IP3/calcium dysregulation. Our data provide a mechanistic explanation for damage induced by surviving infected cells that serve as viral reservoirs and provide potential targets for interventions to reduce the devastating consequences of HIV within the brain.

The unique biological characters of vascular smooth muscle cells in aging-related vascular pathologies. Hongyu Qiu (hqiu@arizona.edu), Internal Medicine & Clinical Translational Sciences, Translational Cardiovascular Research Center, College of Medicine-Phoenix, University of Arizona, Phoenix, Arizona, USA.

Aging-related vascular diseases remain significant contributors to morbidity and mortality due to challenges in clinical treatment. Vascular smooth muscle cells (VSMCs) are recognized as crucial contributors to aging-related vascular pathologies and players of regional different vascular functions. The alterations in VSMC phenotypes and their molecular profiles serve as biomarkers reflecting the progression of vascular diseases and the efficacy of drug treatments. Through integrated in vivo, ex vivo, and in vitro studies, we have demonstrated that VSMCs contribute to aortic stiffening in aging and hypertension independent of neurohormonal effects, endothelial cells, or extracellular matrix. Furthermore, employing advanced bioengineering techniques such as atomic force microscopy (AFM) and 3D reconstituted tissue model, we unveiled unique biological characteristics of aging VSMCs, including increased intrinsic mechanical properties, maladaptive responses to forces, dysregulation in ECM remodeling and their interaction with ECM. These findings underscore the multifaceted roles of VSMCs in vascular pathologies. Moreover, we identified regional disparities in genomics and functions of VSMCs, which may influence regional arterial functions. Additionally, our studies revealed that pharmacological treatments targeting VSMC-mediated mechanisms could alleviate aortic wall stiffening and hypertension in animal models, highlighting VSMCs as promising therapeutic targets.

Novel molecular and cellular insights of non-canonical renin-angiotensin system in cardiovascular diseases. Sergio Lavandero (slavander@uchile.cl), Advanced Center for Chronic Diseases (ACCDiS), University of Chile, Santiago, Chile & University of Texas Southwestern Medical Center, Dallas, Texas, USA.

The classical or canonical renin-angiotensin system (RAS) regulates cardiovascular function. Angiotensin II (Ang II) is the major effector of the RAS, formed by the action of renin and ACE on substrate precursor peptides. Ang II acts by activating the AT1 receptor. An alternative SRA has been discovered, including angiotensin-(1-7) and angiotensin- (1-9), the proteases ACE and ACE2, and the receptors MAS and AT2R. This new SRA has opposed action to the classic SRA and offers new drug targets for developing the cardiovascular drug. Ang-(1-9) is a peptide produced by ACE2 from angiotensin I, acting through AT2R. This peptide prevents cardiac hypertrophy, cardiomyocyte death, and decreased blood pressure. Its potential pharmacological application is limited due to its short plasma half-life. One strategy for designing protease-resistant peptides is the synthesis of retro-inverse analogs. We synthesized a retro-enantiomer Ang-(1-9) [Ang-(1-9) RE]. This analog exhibited increased chemical stability with in vivo anti-hypertensive, cardioprotective, and anti-hypertrophic activities similar to Ang-(1-9). In summary, we reported the first retro enantiomer for the RAS and opened an avenue for the design of new families of peptidomimetics that could contribute to improving therapy for cardiovascular diseases.
Acknowledgment: FONDAP 1523A0008, FONDECYT 1240443

Stress response in pathological cardiac remodeling. Zhao V Wang (zhaowang@coh.org), Department of Diabetes and Cancer Metabolism, Beckman Research Institute, City of Hope National Medical Center, Duarte, California, USA.

Cardiomyocyte growth is coupled with active protein synthesis. However, whether the unfolded protein response (UPR) transducers directly participate in protein synthesis remains unclear. We employed cardiomyocyte-specific inositol-requiring enzyme 1α (IRE1α) knockout and overexpression mouse models. In addition, neonatal rat ventricular myocytes (NRVMs) were cultured to evaluate the role of IRE1α in vitro. Moreover, ribosome sequencing (ribo-seq) was performed to determine the molecular basis for IRE1α in translational control. IRE1α is required for cell growth in NRVMs under pro-hypertrophy treatment. We further find that IRE1α interacts with eIF4G and eIF3, components of the translation initiation complex. IRE1α facilitates the formation of the translation initiation complex and preferentially initiates the translation of mRNAs with 5' terminal oligopyrimidine (TOP) motifs. IRE1α stimulates the epidermal growth factor receptor (EGFR) translation through an unannotated TOP motif. We further demonstrate the physiological role of IRE1α-governed protein translation by showing that IRE1α is essential for cardiomyocyte growth and cardiac functional maintenance under hemodynamic stress. These studies suggest a non-canonical role of IRE1α in orchestrating protein synthesis, which may have important

implications in cardiac hypertrophy in response to pressure overload and cell growth under other physiological and pathological conditions.

Symposium 3: Beyond the observation limit in biology with single molecule techniques

Mass photometry as novel approach to study oligomerization and structure-function relationship of immunoglobulin binding protein (BiP). Karina New[1] (karina.knew@gmail.com), Miguel Lagos[1], John Young[2], Nathalie Casanova-Morales[3], Roi Asor[2], Zahra Alavi [4], Philipp Kukura[2] Christian A.M. Wilson[1]. [1]Faculty of Chemical and Pharmaceutical Sciences, University of Chile. [2]Department of Chemistry, University of Oxford. [3]Faculty of Liberal Arts, Universidad Adolfo Ibáñez. [4]Department of Physics, Loyola Mount University.

Introduction: Human immunoglobulin binding protein (BiP) is a HSP70 chaperone located in the endoplasmic reticulum with many roles in proteostasis. BiP exhibits ATPase properties and the nucleotide state allosterically effects polypeptide binding. BiP self-associates into multiple oligomeric species that structural studies show to bind in the substrate binding domain (SBD). However, physical properties of higher order species are not well investigated.
Materials and Methods: Mass photometry (MP) assays can be employed to study the stability of BiP monomers in solution and their concentration dependent tendency to assemble into higher-order oligomers. When performed as a function of concentration or ligands, binding parameters such as affinity and dissociation kinetics can be eluded from MP studies. Additional methods were used to corroborate MP findings and augment MP techniques to elude binding parameters.
Results: MP studies reveal similar BiP dimer: monomer ratios in solution in absence of nucleotides and presence of ADP or ATP-γ-S (0.13, 0.21 and 0.11, respectively) but when ATP is added this is reduced (to 0.03). This demonstrates monomer stabilisation effect occurs upon ATP binding. When studied in detail, the dissociation constant of ATP is calculated as 0.1 μM, similar to that determined by other techniques.
It was also observed that ligands differentially altered BiP dimerisation at low or high BiP concentrations. Further study reveals two distinct clusters of K_D values are seen between nM and μM concentration ranges of BiP.
Discussion: We provide functional data to support a BiP self-binding site within the SBD. Our data also suggest that BiP monomers form distinct species of dimers in a concentration dependent manner. BiP dimers do not demonstrate ATPase activity, proposing a mechanism of activation by ATP or substrate mediated monomerisation. Novel MP approaches to monitoring small ligand interactions in oligomer forming proteins, and methods to measure k_{on} and k_{off} parameters are proposed.
Funded by: EMBO scientific exchange grant 9880.

Mechanochemical Regulation of the Talin Mechanosensor. Dr. Rafael Tapia Rojo, Department of Physics, King's College London, London, UK.

Talin is a critical mechanosensitive protein, which crosslinks integrins with the actin filaments, serving as a protein interaction hub that regulates focal adhesion dynamics. Being stretched by the forces transmitted from the extracellular environment and those exerted by the actomyosin machinery, talin's function is largely determined by the mechanical response of its rod domains, which undergo force-dependent conformational changes to regulate signalling events, including binding interactions and post-translational modifications. We have developed novel single-molecule magnetic tweezers instrumentation to study the conformational response of talin mechanosensitive domains under physiologically relevant forces and timescales. Here, I will present recent data showing the intricate conformational dynamics of the talin R3 domain when explored over long timescales, and the molecular mechanism underlying the interaction and activation of its main binding partner, vinculin.

Force-triggered phosphorylation of the focal adhesion kinase modulates protein folding dynamics. Dr. Greta Griniute, Department of Physics, King's College London, London, UK.

The focal adhesion kinase (FAK) operates as a force-sensing kinase, localized to focal adhesions (FAs) through interactions between its focal adhesion targeting (FAT) domain and paxillin. FAK's ability to disassemble FAs and regulate cell motility depends on Src-mediated phosphorylation of Y925 on FAT, thereby disturbing paxillin binding. However, previous bulk biochemical experiments have demonstrated the elusive nature of Y925 phosphorylation when FAT is in its native folded state. This suggests the necessity of a conformational change to facilitate Src binding, enabling subsequent phosphorylation of Y925. Here, we employ single-molecule magnetic tweezers force spectroscopy and Molecular Dynamics (MD) simulations to investigate FAT-paxillin binding and real-time phosphorylation, uncovering mechanosensing capabilities affecting the Y925 site exposure. FAT exhibits a sharp force-response characterized by classic all-or-none two-state folding dynamics with occasional reversible misfolding events. Although the LD2 motif of paxillin interacts weakly with FAT, LD2 and LD4 bind cooperatively, resulting in a significantly increased affinity under force. Our single-molecule assay demonstrates that phosphorylation of Y925 is a cryptic event, where force triggers this Src-mediated post-translational modification. Phosphorylated FAT shows a markedly altered folding response, with decreased mechanical stability and the emergence of an intermediate state in the folding pathway. MD simulations corroborate our experimental findings, suggesting that the intermediate state corresponds to a metastable conformation where the helix harboring phosphorylated Y925 is detached from the folded bundle of FAT. Overall, our work showcases the intricate role of mechanical

forces in modulating protein dynamics and signaling events in mechanosensitive proteins to eventually regulate biological responses.

Titin Atlas: Mechano-Physiology of the Largest Molecule in Muscles. J. Andrés Rivas Pardo[1] (jaime.rivas@umayor.cl), Pablo Berrios[1], Ivana Orellana[1]. [1]Mechano Biology Group, Microbe Genomics lab, Center for Genomics and Bioinformatics, Universidad Mayor, Santiago, Chile.

Introduction: Titin is the largest protein in the human body. Constituted by more than 300 Immunoglobulin-like (Ig) domains, titin determines the passive elasticity of muscle tissue. Current theories of muscle contraction propose that the power stroke of a myosin motor is the sole source of mechanical energy driving the sliding filaments of a contracting muscle. Nevertheless, emerging evidence supports that titin could contribute to the early stages of muscle shortening through protein unfolding and refolding. Due to the large size of the molecule, *in vitro* and *in silico* mechanical assays have been limited to study only a couple Ig domains or short regions, preventing the complete understanding of titin under force. Through a combination of protein engineering and computational biology, we have implemented an assay for studying the elasticity of large titin segments, enabling us to study titin under physiological forces and evaluate the action of muscle chaperones.
Material and Methods: To characterize the titin I-band region, we have combined *in silico* assays based on steered molecular dynamics and single molecule force spectroscopy. In particular, we have implemented the SpyTag-SpyCather technology for communicating different titin segments and studying them under force.
Results: Our findings suggest that titin rapidly unfolds when single molecules are equilibrated a physiological force, reaching equilibrium positions where unfolded Ig domains coexist with folded domains. Moreover, muscle chaperones contribute to achieving the folded state, suggesting that titin can experience shortening even in the presence of a mechanical force.
Discussion: In conclusion, our studies demonstrate that Ig domain (un)folding reactions in titin occur at physiological forces with a complex contribution of different domains that, together, may provide enough contractile work to the muscle shortening.
Acknowledgments: This work was supported by FONDECYT 1221064

Symposium 4: Molecular Mechanisms of Root Development and Stress Responses in Plants

Role of plant endomembrane trafficking on root architecture and responses to environmental cues. Lorena Norambuena (lnorambuena@uchile.cl). Plant Molecular Biology Centre, Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile.

Plants have the amazing ability of perceive and respond to the ever-changing environment. Under adverse conditions, plants display a variety of mechanisms at molecular and cellular level that translate into physiological processes allowing them to adapt appropriately. Those responses relay on cellular organization and function where the endomembrane trafficking has a pivotal role. Our research has unraveled mechanisms of endomembrane trafficking and its role on developmental and physiological responses in *A. thaliana*. By means of chemical genetics we have described a novel molecular pathway driven by endocytic trafficking that regulates root architecture. Lately, we have driven our attention on the role of the endomembrane trafficking and its regulation of on responses to abiotic and biotic stress conditions. We are unraveling the mechanism underneath by using molecular biology, genetics, genomics, and chemical biology. Such mechanisms would have an extreme relevance on plant physiology particularly to overcome detrimental environmental conditions.
Funding: FONDECYT 1211311 and CeBioCliF ATE220043

Long noncoding RNAs in Arabidopsis root development. Federico D. Ariel (fariel@fbmc.fcen.uba.ar). Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE), CONICET-Universidad de Buenos Aires, Argentina.

With the advent of next generation sequences technologies, a growing number of long noncoding RNAs (lncRNAs) have been identified across plant species. A subset of them have been functionally characterized, both at the molecular and physiological levels. Plant lncRNAs exert their essential functions through various mechanisms, including modifications to chromatin structure, regulation of alternative splicing, fine-tuning of miRNA activity, and control of mRNA translation or accumulation. In our lab, we have characterized several lncRNAs involved in alternative splicing and epigenetics, respectively, participating in different pathways controlling root development. Here, we uncovered the role of lncRNAs interacting with transcription factors (TFs). In *Arabidopsis thaliana*, NF-YA2 and NF-YA10 (Nuclear Factor A2 and A10) are TFs post-transcriptionally regulated by microRNAs, participating in a regulatory hub controlling root architecture. RNA-seq and ChIP-seq approaches served to identify NF-YA2 direct target genes involved in lateral root (LR) development. Furthermore, we searched for NFYA2 potential RNA partners by using RIP-seq and identified a lncRNA named *AtBAMBOO* after its counterpart *PANDA*, which interacts

with mammalian NF-YA. *AtBAMBOO* is dynamically regulated during LR development and *Atbamboo* mutant plants exhibit a NF-YA2-related root developmental phenotype. *Atbamboo* plants share half of its deregulated genes with the NF-YA2 regulome. Furthermore, overexpressing the lncRNA *AtBAMBOO* disturbed NF-YA2 binding to its target promoters suggesting an RNA-based competition of target recognition by NF-YA2 in chromatin. In agreement, a relocalization of NF-YA2 from the nucleus to the cytoplasm was observed upon *AtBAMBOO* overexpression. Hence, the study of lncRNAs at the organismal level underscores their capacity to intricately modulate TF activity throughout targeted developmental processes, thereby fine-tuning regulatory networks.

Understanding signaling pathways governing the polar development of plant root hairs in low-temperature, nutrient-deficient environments. José M. Estevez[1,2]. [1]Fundación Instituto Leloir and IIBBA-CONICET. Av. Patricias Argentinas 435, Buenos Aires, Argentina. [2]ANID - Millennium Nucleus for the Development of Super Adaptable Plants (MN-SAP) and Millennium Institute for Integrative Biology (iBio), Santiago 8331150, Chile. Centro de Biotecnología Vegetal (CBV), Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago, Chile

Plants exposed to freezing and above-freezing low temperatures must employ a variety of strategies to minimize fitness loss. There is a considerable knowledge gap regarding how mild low temperatures (around 10 °C) affect plant growth and developmental processes, even though the majority of the molecular mechanisms that plants use to adapt to extremely low temperatures are well understood. Root hairs (RH) have become a useful model system for studying how plants regulate their growth in response to both cell-intrinsic cues and environmental inputs. Here, in this talk I'll focus on recent advances in the molecular mechanisms underpinning *Arabidopsis thaliana* RH growth at mild low temperatures and how these discoveries may influence our understanding of nutrient sensing mechanisms by the roots. I will explore the molecular basis of this strong RH growth response involving a cell surface receptor-like kinase named FERONIA, cell wall PEROXIDASES, downstream components of the TORC1 pathway and a complex regulation of the gene expression. Although nutrient availability in the soil is one of the key factors for a sustained plant growth, the molecular mechanisms behind the perception and the downstream signaling pathway in the roots are still far to be clear.

Dissecting nitrate responses in time and space. Rodrigo A. Gutiérrez (rgutierrez@uc.cl). ANID Millennium Institute for Integrative Biology (iBio), ANID Millennium Institute Center for Genome Regulation (CRG) and Institute of Ecology and Biodiversity (IEB), Pontificia Universidad Católica de Chile. Av Libertador Bernardo O'Higgins 340, Santiago, Chile.

Nitrate is a nutrient and a potent signal that impacts global gene expression in plants. Regulatory factors controlling spatiotemporal nitrate responses are still largely unknown. In order to address this problem, we assayed nitrate-responsive transcriptome changes in five major root cell types of the *Arabidopsis thaliana* root as a function of time. We found that gene-expression response to nitrate is dynamic and highly localized and identified cell type–specific transcription factor (TF)–target interactions. Among cell types, the endodermis stands out as having the largest and most connected nitrate-regulatory gene network. ABF2 and ABF3 are major hubs for transcriptional responses in the endodermis cell layer. We experimentally validated TF–target interactions for ABF2 and ABF3 by chromatin immunoprecipitation followed by sequencing and a cell-based system to detect TF regulation genome-wide. Validated targets of ABF2 and ABF3 account for more than 50% of the nitrate-responsive transcriptome in the endodermis. Moreover, ABF2 and ABF3 are involved in nitrate-induced lateral root growth. We obtained an unprecedented spatiotemporal resolution of the root response to nitrate and identified important components of cell-specific gene regulatory networks.

Symposium 5: Molecular interactions controlling host-microbe interactions: from microbial pathogenesis to host immunity.

Elucidating the Mechanism of Action of Isoniazid; Implications for new drug development. Dr. William Jacobs Jr. (william.jacobs@einsteinmed.edu), Professor, Department of Microbiology and Immunology, Albert Einstein College of Medicine.

Developed over 70 years ago, Isoniazid is one of the most efficient drugs for the treatment of Mycobacterium tuberculosis (Mtb). Yet, its precise mechanism of killing could not be elucidated until Mtb gene transfer systems were developed. The combination of genetic, biochemical, and X-ray crystallographic analyses provided a consistent model indicating that INH is activated by the catalase-peroxidase KatG, forming a complex with NAD. This complex inhibits the inhA enzyme, essential for the synthesis of mycolic acids, ultimately resulting in cell death. The discovery of mutations conferring INH resistance has further refined our understanding of its complex mechanism. This knowledge has been instrumental in developing molecular diagnostic tests to detect drug resistance, aiding in more effective TB treatment and prevention.

Vitamin D receptor protects intestinal barriers against dysbiosis and tumorigenesis. Jun Sun[1,2] (junsun7@uic.edu), Yongguo Zhang[1], Yinglin Xia[1]. [1]University of Illinois Chicago UIC), Department of Medicine. [2]UIC Cancer Center, Chicago, IL, USA.

Introduction: Vitamin D and Vitamin D receptor (VDR) have novel functions beyond the classical roles in bone development. VDR activates innate immunity and affects intestinal development patterns. Low expression of VDR and dysfunction of vitamin D/VDR signaling are reported in patients with inflammatory bowel disease. We have reported VDR in shaping human microbiome. The intestinal epithelium cells (IECs) are essential in barrier function, structural function, and host defense. The aim of this presentation is to determine intestinal VDR regulation of microbiome and intestinal barriers in cancer development.
Methods: We used an azoxymethane/dextran sulfate sodium–induced cancer model in intestinal VDR conditional knockout (VDR^{DIEC}) and IECVDR-over expressing mice, cell cultures, stem cell–derived colonoids, and human colon cancer samples.
Results: VDR^{DIEC} mice had higher numbers of tumors, compared to the VDR^{LoxP} mice. We found more bacterial endotoxin lipopolysaccharide (LPS) in VDR^{DIEC} mice than in VDR^{LoxP} mice, especially in tumor groups. Intestinal barrier function was weakened by reduced tight junctions in VDR^{DIEC} mice. Bacteroides fragilis was translocated to and enhanced in tumors. We identified tight junction CLDN-5 as a downstream target of VDR. There were Vitamin D response element binding sites in the Claudin-5 promoter required for vitamin D3-induced Claudin-5 expression. Conditional epithelial VDR overexpression protected against the loss of Claudin-5 in tumorigenesis.
Discussion: We highlight the VDR-dependent enhancement of *Bacteroides fragilis* and reduced barrier in colon cancer. We provide insights into the mechanism of VDR dysfunction leading to dysbiosis and dysfunction of barrier in tumorigenesis. Understand the mechanisms of the VDR-microbiome-dependent intestinal barriers will advance the cancer prevention and treatment.

Gastrokine-1 and protection from inflammatory bowel disease. David Boone[1,2] (dboone@nd.edu), Theo Reed[2], Arpitha Mysore Rajeskera[2], Morgan Hiller[2], Katelyn Haase[2], Alvaro Torres-Huerta[1]. [1]University of Indiana, School of Medicine. [2]University of Notre Dame.

Introduction: Inflammatory bowel diseases (IBD) are chronic inflammatory disorders of the gut. The causes of IBD are unknown but a current paradigm is that IBD results from a combination of genetic and environmental factors that cause excessive inflammation leading to loss of intestinal function. Gut microbes are likely key mediators of the pathology of IBD, responding to host signals, environmental cues and the mucosal immune system. We have found that a protein made in the gut, called gastrokne-1 (Gkn1) is important for

protection against IBD. Thus, our goal is to understand how Gkn1 functions to provide protection from IBD.
Materials and Methods: Mice lacking Gkn1 (Gkn1$^{-/-}$) were compared to WT littermates in models of colitis (IBD of the colon). recombinant Gkn1 protein was generated to study the interaction oof Gkn1 with gut microbes.
Results: Gkn1$^{-/-}$ mice were more susceptible to chemically induced colitis and to gut fibrosis compared to WT littermates. Gkn1$^{-/-}$ mice exhibited an overgrowth of *Enterococcus faecalis* in their distal gut. Recombinant Gkn1 inhibited amyloid formation by the microbial amyloid peptide CsgA. Consistent with this anti-amyloid function, Gkn1 inhibited microbial biofilm formation. Gkn1$^{-/-}$ mice were less able to clear infection by the biofilm forming microbes *E. coli* (LF82) and *Citrobacter rodentium*. Feeding Gkn1 to Gkn1$^{-/-}$ mice ameliorated colitis.
Conclusion: Gkn1 is required for protection from chemically and microbially induced colitis and feeding Gkn1 may be a potential therapy for IBD.
Acknowledgement: NIH-NIDDK/1R01DK124304, Indiana CTSI NIH-NCATS UM1TR004402.

Restoring dendritic cell function to elicit protective immunity against herpetic skin disease. Dr. Pablo González (pagonzam@uc.cl), Millennium Institute on Immunology and Immunotherapy, Pontificia Universidad Católica de Chile.
Herpes simplex virus (HSV) infections are highly prevalent in the human population, eliciting both mild and severe diseases, such as recurrent herpetic skin lesions to life-threatening encephalitis. Importantly, these pathogens infect immune cells, such as dendritic cells (DCs) to evade the host antiviral response. Indeed, HSVs hamper DC presentation of viral antigens, maturation and migration to lymph nodes, interfering with virus-specific T cell activation. Importantly, we have identified cellular processes that if activated or inhibited during HSV infection can re-establish DC function and elicit immune cell activation and antiviral immunity.

The Importance of European Pharmacopoeia Quality Control Standards/Guidelines in Vaccine Development. Dr. Ioannis Rabias1 (ioannisrabias@pasteur.gr), Head of Quality Control Department, Qualified Person of the Vaccine Unit of the Hellenic Pasteur Institute, Member of the European Pharmacopoeia Group of Experts on Human Vaccines. 1Quality Control Department, Hellenic Pasteur Institute, Athens, Greece.

The European Directorate for the Quality of Medicines and Healthcare (EDQM) is the Directorate of the Council of Europe responsible for developing legally binding quality standards for good quality medicines. Its establishment dates back to 1964, when the international convention for the elaboration of a European Pharmacopoeia was approved by the Council of Europe with the vision of creating a common European Pharmacopoeia.
The European Pharmacopoeia is a single reference project for the quality control of medicines in 38 Member States as well as in the European Union (EU). Officially published

standards provide a legal and scientific basis for quality control during development, production and marketing processes. They concern the qualitative and quantitative composition and the tests to be carried out on medicinal products, on the raw materials used in the manufacture of medicinal products and on the intermediate products of the composition.
Therefore, all manufacturers of medicines and / or substances for pharmaceutical use must apply these quality standards in order to market their products in the signatory States.
The scope of Quality Control Department of the Hellenic Pasteur Institute is to assure that vaccines and other pharmaceutical products conform to specifications in compliance with the current European pharmaceutical legislation. Quality Control provides reliable laboratory analyses based on principles of good manufacturing practice (EU-GMP), in an independent and impartial way, so as to ensure the high quality, accuracy and reliability of the analytical results with a view to the protection of public health, the excellent service and satisfaction of the requirements of its clients and the overall effective fulfillment of its mission. Quality Control Department is an independent unit and is comprises of 3 quality control laboratories and a sterile area with grade zones B/C/D.

Symposium 6: Plant System Biology: from molecules to the ecosystem.

Single-plant omics: profiling individual plants in a field to identify processes affecting yield. Steven Maere[1,2] (steven.maere@psb.vib-ugent.be), Michael Van de Voorde[1,2], Sam De Meyer[1,2], Daniel Felipe Cruz[1,2], Stijn Hawinkel[1,2], Tom De Swaef[3], Peter Lootens[3], Jolien De Block[1,2], Tom Van Hautegem[1,2], Dirk Inzé[1,2], Hilde Nelissen[1,2], Isabel Roldán-Ruiz[3]. [1]Department of Plant Biotechnology and Bioinformatics, Ghent University, Technologiepark 71, 9052 Gent, Belgium. [2]VIB Center for Plant Systems Biology, Technologiepark 71, 9052 Gent, Belgium. [3]Plant Sciences Unit, Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Caritasstraat 39, 9090 Melle, Belgium.

Introduction: Historically, processes influencing plant phenotypes have been studied intensively under controlled laboratory conditions. However, the results of such controlled lab studies often do not translate well to more complex field settings.
Materials and Methods: To help close this lab-field gap, we developed a new experimental setup to study the wiring of plant traits directly in the field, based on omics profiling, micro-environmental profiling and phenotyping of individual plants of the same genetic background grown in the same field.
Results: We used this single-plant omics strategy on winter-type rapeseed (*Brassica napus*) and built machine learning models predicting various phenotypes of field-grown rapeseed plants from their autumnal leaf gene expression and environmental data layers such as soil nutrient profiles and microbiomes at single-plant resolution. We find that autumnal leaf gene expression has predictive power for both autumnal leaf phenotypes and final yield phenotypes in spring. Many of the top yield predictors are linked to developmental

processes known to occur in autumn in winter-type *B. napus* accessions, such as the floral transition, indicating that the yield potential of winter-type rapeseed is influenced by autumnal development. We applied methods from the single-cell field on our single-plant data to further unravel these developmental effects.
Discussion: Our results show that the single-plant omics setup can be used to identify processes influencing crop yield in the field.
Acknowledgment: FWO 1146319N, VLAIO HBC.2019.2814.

Gene regulatory networks of the sulfate deficiency response in *Solanum lycopersicum.* Elena A. Vidal[1,2] (elena.vidal@umayor.cl), José David Fernández[1,2], Joaquín Medina[3], Tomás Matus[4], José M. Álvarez[2,5], Javier Canales[2,6]. [1]Centro de Genómica y Bioinformática, Universidad Mayor, Chile. [2]Instituto Milenio de Biología Integrativa iBio, Chile. [3]Centro de Biotecnología y Genómica de Plantas, UPM-CSIC-INIA, Spain. [4]Instituto de Biología Integrativa de Sistemas I[2]SysBio, Spain. [5]Centro de Biotecnología Vegetal, Universidad Andrés Bello, Chile 6Instituto de Bioquímica y Microbiología, Universidad Austral, Chile.

Introduction: Deficiency of sulfate (S), a relevant nutrient for plant growth, results in diminished photosynthetic rates, amino acid contents and disrupted acquisition of other nutrients. Plants modulate their physiology and metabolism in response to S deficiency, optimizing its acquisition, assimilation, and utilization. These changes are caused in part by a reprogramming of the plant's transcriptome. While these changes in gene expression have been investigated in the model plant *Arabidopsis*, our understanding of the regulatory mechanisms governing the sulfate response in relevant crops such tomato remains limited.
Materials and Methods: To gain molecular insights into the S deficiency response in tomato, we performed a transcriptomic analysis in tomato. To identify transcription factors (TFs) controlling the response, we generated reference gene regulatory networks employing GENIE3. We determined a potential key TFs responsible for regulating S deficiency-responsive genes, of which we further analyzed the function of one of them.
Results: We found tomato plants are highly responsive to S deficiency, compared to other crops and *Arabidopsis*. Network analysis determined that the main regulator of this response is *Sl*EIL3, a close homolog of SLIM1, pivotal regulator of S transport and metabolism in *Arabidopsis*. *Sl*EIL3 controls genes related to sulfate transport and metabolism, as well as other genes related to stress responses and hormonal responses in tomato. Furthermore, *Sl*EIL3 overexpression in *Arabidopsis* promotes plant growth and increases the sulfate contents of the plants.
Discussion: We identified the first TF controlling the S deficiency response in tomato, implicating this TF in the control of important genes for S homeostasis, as well as other relevant processes.
Acknowledgements: ANID-FONDECYT 1211130 1210389, 1230833, ICM-ANID iBio ICN17 022, FOVI230159, Beca Doctoral ANID 21230478.

Opposite Forces: How Plants Integrate Stress and Nutritional Signals. José Miguel Alvarez[1,2] (jose.alvarez.h@unab.cl). [1]Centro de Biotecnología Vegetal, Universidad Andrés Bello, Chile. [2]Agencia Nacional de Investigación y Desarrollo – Millenium Science Initiative Program, Millenium Institute for Integrative Biology (iBio), Santiago, Chile.

Introduction: Drought and nitrogen (N) availability are key factors limiting plant growth and crop productivity. Understanding how plants balance stress response with growth programs through transcriptional networks is crucial. While the molecular mechanisms for N or drought signaling have been studied, the combined response to N and drought signals at the molecular level is less understood.
Materials and Methods: A combinatorial experiment was conducted to vary N and drought levels simultaneously in *Arabidopsis thaliana* and *Solanum lycopersicum* (tomato) to characterize phenotypic and transcriptomic responses. We performed differential gene expression and gene regulatory network analyses to uncover the regulatory mechanisms and relevant transcription factors (TFs) modulating gene responses. The validation of TFs was performed by ChIP-seq to capture TF binding and a cell-based assay to capture direct transcription factor TF regulation genome-wide.
Results: We found significant overlaps in gene regulation for N, ABA, and drought treatments. N and drought-regulated genes showed a strong negative correlation, indicating opposing signals. Network-based analysis reveals high interconnectivity between regulatory circuits controlling N and drought responses. N-related TFs influence the expression of stress TFs and vice versa.
Discussion: These findings reveal convergent regulatory circuits in plant responses to conflicting N and drought signals. They advance our understanding and enable genetic modifications to enhance plant development and stress resistance, which are crucial for optimizing crop yield and sustainable agriculture.
Acknowledgment: ANID Fondecyt Regular 1210389, Instituto Milenio de Biología Integrativa (iBio) ICN17_022, and National Science Foundation NSF IOS-1840761 USA.

Phased, secondary siRNAs in plant reproduction and other pathways. Blake Meyers, The Genome Center, and Department of Plant Sciences, University of California – Davis.

In plants, 21 or 22-nt miRNAs or siRNAs typically negatively regulate target genes through mRNA cleavage or translational inhibition. Heterochromatic or Pol IV are 24-nt and function to maintain heterochromatin and silence transposons. Phased “secondary” siRNAs (phasiRNAs) are generated from mRNAs targeted by a typically 22-nt “trigger” miRNA, and are produced as either 21- or 24-mers via distinct pathways. Our prior work in maize and rice demonstrated the temporal and spatial distribution of two sets of “reproductive phasiRNAs”, which are extraordinarily enriched in the male germline of the grasses. These two sets are the 21-nt (pre-meiotic) and 24-nt (meiotic) siRNAs. Both classes are produced

from long, non-coding RNAs, generated by hundreds to thousands of loci, depending on the species. These phased siRNAs show striking similarity to mammalian piRNAs in terms of their abundance, distribution, distinctive staging, and timing of accumulation, but they have independent evolutionary origins. The functions for these small RNAs in plants remain poorly characterized. I will describe our recent work investigating the functions of plant phasiRNAs and their roles in modulating traits of agronomic importance in plants, including male fertility.

Symposium 7: Biochemistry and Molecular Biology of Fruit Ripening and Plant Metabolites

***Fragaria chiloensis* fruit as a model for studying ripening events.** María Alejandra Moya-León[1] (alemoya@utalca.cl.), Macarena Zamorano-Curaqueo[1,2], Felipe Valenzuela[1], Raúl Herrera[1]. [1]Functional Genomics, Biochemistry and Plant Physiology Group, Instituto de Ciencias Biológicas, Universidad de Talca. [2]Doctorado en Ciencias, mención Ingeniería Genética Vegetal, Universidad de Talca.

Introduction: *Fragaria chiloensis* is a Chilean native species that softens intensively during its ripening. Its softening is related to cell wall disassembly due to the participation of cell wall degrading enzymes such as *FchXTH1*, *FchRGL*, *FchEXP2*, *FchPL* and *FchPG*. However, the molecular machinery involved in the transcriptional regulation has not been clarified. On the other hand, the expression of FchAGL9 and FchSHP, two MADS-box transcription factors (TF) belonging to different subfamilies, increments during softening. Therefore, their participation in softening of *F. chiloensis* fruit was analyzed.
Materials and Methods: *Fragaria chiloensis* fruits were agro-infiltrated with overexpression vectors containing the *FchAGL9* or *FchSHP* sequences, or the empty vector (EV). Fruit tissues were collected after two days, frozen and stored at -80°C. Total RNA was extracted and cDNA synthesized. The level of transcripts codying for cell wall degrading genes was quantified by RT-qPCR. TF characterization was complemented through EMSA, bimolecular fluorescence complementation (BiFC), yeast two-hybrid (Y2H), luciferase-dual assays and protein modelling.
Results: FchAGL9 and FchSHP are expressed only in flower and fruit tissues, rising as the fruit softens. EMSA assays demonstrated that FchAGL9 binds to CArG sequences of RIN and SQM, meanwhile FchSHP interacts only with RIN. BiFC and Y2H assays confirmed FchAGL9-FchAGL9 and FchAGL9-FchSHP interactions. Hetero-dimer structure was built through homology modeling concluding that FchSHP monomer binds to DNA. Luciferase-dual assays indicated that FchAGL9 transactivates *FchRGL* and *FchPG´*s promoters, meanwhile FchSHP transactivates those of *FchEXP2*, *FchRGL* and *FchPG*. Over-expression of *FchAGL9* in C2 *F. chiloensis* fruit rises *FchEXP2* and *FchEXP5* transcripts, meanwhile the over-expression of *FchSHP* also increments *FchXTH1* and *FchPL*; in both cases there is a down-regulation of *FchRGL* and *FchPG*.

Discussion: These results provide evidence of FchAGL9 and FchSHP participating in the transcription regulation associated to *F. chiloensis´*s softening.
Acknowledgment: FONDECYT 1210948 and ANILLO ACT210025 grants.

Regulation of Carotenoid Biosynthesis and Salt Stress Tolerance given by DcPSY2 and Abscisic Acid Responsive Transcription Factors in Carrot. Stange, Claudia[1] (cstange@uchile.cl), Quiroz-Iturra, Luis Felipe [1,4]; Simpson, Kevin [2,4]; Acevedo, Orlando [3,4], González-Calquín, Christian[1]. [1]Plant Molecular Biology Centre, Department of Biology, Faculty of Sciences, Universidad de Chile, Las Palmeras 3425, Ñuñoa, Santiago, Chile. [2]Genetics & Biotechnology Lab, Plant & AgriBiosciences Research Centre (PABC), Ryan Institute, University of Galway, University Road, H91 REW4 Galway, Ireland. [3]Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Alameda 340, Santiago, Chile. [4]Laboratorio de Biología Vegetal e Innovación en Sistemas Agroalimentario, Instituto de Nutrición de los Alimentos (INTA), Universidad de Chile, El Líbano 5524, Macul, Santiago, Chile.

Introduction: Carotenoids are essential for plant functions such as photo-protection and photosynthesis, and they serve as precursors for abscisic acid (ABA), a hormone that mediates responses to abiotic stress. Phytoene synthase (PSY) is a key enzyme in regulating carotenoid biosynthesis in plants. In carrots (*Daucus carota*), which accumulate high levels of carotenoids in their roots, two *PSY* genes, *DcPSY1* and *DcPSY2*, have been identified. Notably, *DcPSY2* expression is induced by salt stress and ABA. We characterized a promoter fragment of *DcPSY2*, revealing three ABA responsive elements (ABRE) and several ALFIN-like elements. ALFIN transcription factors play roles in root development and stress responses. Further analysis identified three AREB/ABF (DcAREB) and DcALFIN4 and DcALFIN7 transcription factor candidates in the carrot transcriptome.
Methods: By means of bioinformatics, qRT, monohybrid (YH1), overexpression of candidate genes, and salt stress assays we characterized the mechanism by which DcPSY2 is regulated by DcAREB3 and DcALFINs thus enhancing salt stress tolerance and carotenoid synthesis.
Results: *DcAREB3* and *DcALFIN4* and *DcALFIN7* expression is specifically induced by ABA or salt stress in carrot roots. These genes encode nuclear proteins that activate transcription in yeast and bind to the *DcPSY2* promoter. Transgenic *N. tabacum* or *A. thaliana* plants expressing *35S:DcPSY2: GFP* and *35S:DcALFIN4: RFP* exhibited higher survival rates under salt stress, which correlated with increased carotenoid levels. This increase in stress tolerance was associated with elevated expression of carotenogenic and abiotic stress-related genes, and higher ABA levels.
Discussion: In conclusion, DcPSY2 and related transcription factors such as DcAREB3, and DcALFIN4 play significant roles in mediating stress responses in plants. The findings support the potential of these genes in enhancing stress tolerance through genetic

engineering, highlighting their importance in carotenoid biosynthesis and plant stress resilience.
Funding: Fondecyt1221399, Anillo PIA ATE220043.

High-value polysaccharides characterized in Chilean papaya mucilage waste. Susana Saez-Aguayo[1,3] ,Dayan Sanhueza[1,3], Pablo Sepúlveda-Orellana[1,3], Raúl Herrera[2,3] and María Alejandra Moya-León[2,3]. [1]Centro de Biotecnología Vegetal, Universidad Andrés Bello, Santiago, Chile. [2]Instituto de Ciencias Biológicas, Universidad de Talca, Talca, Chile. [3]Proyecto Anillo ACT210025 CHICOBIO.

Introduction: The agroindustry generates significant organic waste, including fruits and vegetables rich in non-digestible carbohydrates primarily found in plant cell walls (CW). The composition of these CWs varies across plant species, providing diverse structures with various health benefits, such as cholesterol reduction and anti-inflammatory properties. Inside Chilean papaya fruits, a mucilage surrounds the seeds and is typically discarded during fruit processing. The structure of this mucilage has not been characterized, making the aim of this work to explore and characterize the mucilage structure of papaya seeds.
Materials and Methods: For cytological approaches, mature seed sections were obtained after seed fixation and resin embedding, followed by staining with toluidine blue and CW-antibodies to observe mucilage structure. Staining of the mucilage seed using a direct method, or seeds treated with EDTA and $CaCl_2$, along with Ruthenium red, was conducted to visualize mucilage layers and determine their adherence to the seed surface. Biochemical analyses using HPLC, colorimetric assays, fractionation, and pectin domain separation by a size exclusion chromatography column were used to separate and determine the monosaccharide composition of papaya mucilage.
Results: Papaya mucilage polysaccharides constitute nearly 20% of seed weight. Biochemical analyses revealed that the mucilage is enriched in galacturonic acid, suggesting the presence of a homogalacturonan (HG) domain. The presence of HG was confirmed by antibody labeling and Ruthenium red staining. Further biochemical analysis of fractionated pectin from the papaya mucilage demonstrated a high level of highly methylesterified HG (>75%). Additionally, the treatment of seeds with EDTA and $CaCl_2$ clarified the role of homogalacturonan methylation for mucilage adhesion.
Discussion: HG is renowned for its beneficial health properties and its applications in the food industry. The identification of homogalacturonan-enriched mucilage in papaya seeds underscores its industrial potential, offering a valuable nutritional use for this previously discarded industrial waste.
Acknowledgment: ANILLO ACT210025 grant.

Single-cell decoding of cellular lineages and the regulators giving rise to plant exceptionalism. Matias Kirst[1,2]. [1]School of Forest, Fisheries and Geomatic Sciences, University of Florida, Gainesville, FL, USA. [2]Genetics Institute, University of Florida, Gainesville, FL, USA.

Throughout evolution, certain plant species have developed remarkable traits that give them unique advantages under biotic and abiotic stress conditions. These traits range from the ability to extract nickel from the soil for defense against insect herbivory, to forming symbiotic relationships with nitrogen-fixing bacteria to obtain atmospheric nitrogen, and adapting photosynthesis to withstand water scarcity through crassulacean acid metabolism (CAM). Uncovering the genetic innovations behind these traits could revolutionize crop sustainability and productivity. However, tracing the cellular origins and regulatory mechanisms of these traits has been a formidable challenge—until the advent of single-cell genomics. Our team has pioneered methods to profile single-cell transcriptomes by isolating and analyzing gene expression in solid plant tissues. Additionally, we've developed a web application that streamlines bioinformatics tools for single-cell genomics data analysis. We are leveraging these technologies to explore plant development processes, such as nodule formation in *Medicago truncatula* roots and the facultative CAM photosynthesis. Another focus is the bioengineering of robust carbon products into roots for long-term storage. In this review, I will present our latest research on reconstructing the developmental pathways of differentiating cells and identifying the genes that govern these processes. Our findings aim to enhance our understanding of plant developmental biology and open new avenues for agricultural innovation.

Symposium 8: Challenges and projections of structural biology in South America

Amino acid homeostasis in *M. tuberculosis*: a journey towards integrative structural biology. María Natalia Lisa[1] (lisa@ibr-conicet.gov.ar). [1]Laboratorio de Microbiología Estructural y Biodiseño, Instituto de Biología Molecular y Celular de Rosario (IBR, CONICET-UNR), Argentina.

The eukaryotic-like S/T protein kinase PknG controls the metabolism and virulence of *M. tuberculosis* through phosphorylation of the regulator GarA, which in turn modulates, by phospho-independent mechanisms, three metabolic enzymes that use 2-oxoglutarate as a substrate. Over the past 10 years, we have contributed to elucidating the molecular basis of the PknG-dependent signal transduction pathway present in pathogenic bacteria as well as in free-living species. The studies carried out have combined techniques of structural biology, biochemistry and biophysics, as well as microbiological work and genetic editing of bacteria. Such multidisciplinary approaches, essential to arrive at more comprehensive answers to the biological questions posed, constitute a challenge for scientists in the South. In addition to commenting on the latest advances in the specific topic, I will present the

CEBEM network (Mercosur Center for Structural Biology) as a regional tool to gain access to cutting-edge technology in Integrative Bioimaging.
Acknowledgments: Agencia I+D+i, CONICET, Chan Zuckerberg Initiative

Single-Particle Analysis and in Situ Cryo-ET: Glutaminase Filaments as a Case Study and an Emerging Opportunity in South America. Dr. Andre Ambrosio (andre@ifsc.usp.br), Instituto de Física de São Carlos, Universidad de São Paulo – Brazil.

Abstract: This presentation explores the synergy of cryo-electron microscopy techniques, focusing on the integration of single-particle analysis (SPA) and cryo-electron tomography (cryo-ET) to uncover novel insights into the structure and function of glutaminase filaments. By combining these approaches with complementary fields such as biophysics, biochemistry, and cell biology, we aim to provide a comprehensive understanding of glutaminase mechanisms, revealing unexpected functional roles that challenge current models of enzyme regulation. Additionally, I will highlight ongoing collaborative efforts to establish a state-of-the-art cryo-EM multiuser facility in São Carlos, designed to serve as a regional hub to foster accessibility to these advanced techniques in structural biology.
Funding: FAPESP, CNPq & IFSC.

Rational design of nanobodies for TRP ion channel modulation. González-Nilo, F. D. (fernando.gonzalez@unab.cl), Araya, I., Diaz, I., Marquez, V., Olivares, P., Yevenes, A. Center for Bioinformatics and Integrative Biology, Universidad Andrés Bello.

The rational design of specific nanobodies for ion channel modulation emerges as an innovative strategy with significant implications in both fundamental research and therapeutic applications. These nanobodies can selectively bind to specific subunits of ion channels, such as voltage-dependent calcium channels associated with β1 subunits, allowing precise modulation of channel activity. In this study, we have focused on developing nanobodies capable of modulating TRPM4 channels. TRPM4-specific nanobodies could potentially modulate channel activity, which may be beneficial in reducing reperfusion injury in stroke models, among other pathologies.
Furthermore, nanobodies hold additional applications in immunodetection and structural studies, providing detailed insights into the conformational states and mechanisms of activation and inhibition of ion channels. In this context, we have developed an efficient, rational design strategy for nanobodies, integrating existing PDB data with machine learning methods and novel artificial intelligence-based strategies. Simultaneously, we have explored the possible activation mechanism of TRPM4 through site-directed mutagenesis and molecular simulation strategies, unveiling putative TRPM4 epitopes that could enable more selective modulation compared to traditional drugs. This selectivity is crucial for developing more effective treatments with fewer side effects.
Acknowledgments: This work was supported by FONDECYT project 1221498.

Determination of protein structures in Chile and their contribution to functional and evolutionary studies. Víctor Castro-Fernández (vcasfe@uchile.cl), Nicolás Fuentes-Ugarte, Sebastián M. Muñoz, Isaac Cortés-Rubilar, Martín Pereira-Silva, Andrés Urrutia Santana, Sixto M. Herrera, Felipe Gonzalez-Ordenes, Gabriel Vallejos-Baccelliere, Victoria Guixé. Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

The determination of protein structures is crucial to the understanding of molecular mechanisms and, together with evolutionary analysis, allows us to generate an insight into how molecular mechanisms adapt over time. In recent years, the emergence of structure prediction methods based on artificial intelligence and methodologies such as the reconstruction of ancestral proteins have made it easier to access structural and evolutionary information. However, experimental evidence remains key to guide and validate in sillico predictions. This talk discusses three investigations carried out in Chile that integrate these approaches to answer questions in the evolution of enzyme function and regulation, as well as access to structural information of a biomedical-relevant protein.
The first study focuses on the vitamin kinase family, where X-ray crystallography allowed us to identify critical residues for catalysis, contributing to the understanding of the catalytic mechanism of the condensation of two phosphoryl groups. In the second case, crystallographic information allowed us to identify the allosteric site for AMP in bifunctional PFK/GK enzymes of methanogenic archaea and, using alphafold3, to analyse the evolution of this key metabolic regulation in the sugar metabolism of methanogenic organisms.
Finally, *Concholepas concholepas* haemocyanin (CCH), a protein of biomedical importance that is proposed as an adjuvant in vaccines and as a key principle in the treatment of some cancers. The structural information obtained by X-ray crystallography and electron cryo-microscopy of this protein has allowed us to provide a detailed view of the oxygen binding sites, unusual glycosylations and to propose the architectural organization of the polypeptides for this heterodidecameric protein (20 chains). These studies are intended to show the importance of complementing different structural and prediction techniques through studies carried out in Chile.
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Symposium 9: Systems Biology Centers for the development of research and innovation in the fields of mining, agroindustry and health

SYSTEMIX: Systems Biology Center for the study of extremophile communities from mining tailings. Mauricio Latorre [1,2,3] (mauricio.latorre@uoh.cl).[1]Laboratorio de Bioingeniería; Instituto de Ciencias de la Ingeniería; Universidad de O'Higgins. [2]Centro de biología de sistemas para el estudio de comunidades extremófilas de relaves mineros (SYSTEMIX), Universidad de O'Higgins, Rancagua, Chile. [3]Laboratorio de bioinformática y expresión génica, INTA, Universidad de Chile, Santiago, Chile.

The Cauquenes tailings, located in the O'Higgins Region, is currently the oldest copper tailings deposit in the world. In this context, the identification and characterization of the extremophilic microorganism communities inhabiting the Cauquenes tailings will provide valuable information about the structure of these communities and how they have maintained or changed over time. For these reasons, through the integration of diverse capabilities of national and international researchers, this project aims to lay the foundation for the creation of a Systems Biology Center for the study of communities inhabiting mining tailings, named SYSTEMIX. Our research objectives are: i) Characterization of the structure of extremophilic communities; ii) Identification and validation of the metabolic potentials of the communities and their members; iii) Cataloging and classifying the obtained information through the development of a genomic database of the collected strains; and iv) Applications in the field of biotechnology. With a strong regional commitment and from a multidisciplinary and comprehensive perspective, our project will generate valuable molecular, genomic, and phenotypic information about microorganisms from extreme environments, data that will be fully available to the Chilean scientific community to promote new bridges of collaboration and national and international development.
Funded by: ANID ANILLO ACT210004, Fondecyt Regular 1230194.

A Journey of the molecular epidemiology and genomics approach in *Staphylococcus* and *Enterococcus* in South America. Diana Panesso-Botero[1,2,3,4]. [1]Molecular Genetics and Antimicrobial Resistance Unit, Universidad El Bosque, Bogota, Colombia. [2]Division of Infectious Diseases and Department of Medicine, Houston Methodist Hospital, Houston, TX USA 77030. [3]Department of Medicine, Weill Cornell Medical College, New York, NY. [4]Departament of Pharmacy Practice and Translational Research, University of Houston, Houston, Texas, United States.

Staphylococcus aureus is a significant pathogen responsible for a range of diseases, from mild skin soft tissue infections to severe, life-threatening conditions. Multicenter studies can help to investigate the molecular epidemiology and phylogenomics of *Staphylococcus aureus* across Latin American countries. By employing sequencing techniques, the genetic diversity, transmission patterns, and antimicrobial resistance profiles of SAB isolates has been evaluated. In Chile, we have identified predominant clonal lineages and notable resistance mechanisms, providing crucial insights into local and regional epidemiological patterns. The phylogenomic analysis revealed both intra- and inter-hospital transmission events, highlighting the role of healthcare networks in the dissemination of high-risk clones. *Enterococci* are natural residents of the human gastrointestinal microbiota. *Enterococci* exhibit significant genomic plasticity, which enhances their ability to adapt to antimicrobial challenges. Healthcare-associated isolates of *E. faecium* are resistant to ampicillin, with vancomycin resistance varying from the Latin America countries. Bacterial whole-genome sequencing has proven highly effective in characterizing the epidemiology of multidrug-resistant organisms, identifying outbreaks, and gathering data for public health initiatives.

Using a genomic approach the vancomycin-resistant enterococci can be divided into two clades, a healthcare-associated clade A and a commensal clade B. In Latin America, the clade A2 is not a separate group but rather a series of branches along the evolutionary path of clade A.
Funded by: ANID ANILLO ACT210004, Fondecyt Regular 1230194.

BIOSAV-UOH: Systems biology center for plant health. Lorena Pizarro (lorena.pizarro@uoh.cl)[1,2,3], Ernesto San Blas[1,6], Humberto Aponte[1,7], Karen Mesa[1], Uri Aceituno-Valenzuela [1,2], Brynelly Bastidas[1,2,6], Patricia Morales-Montero[1,2,6], Mariela González [1,2], Gabriel Gálvez[1,3,4], Jaime Ortega[1,3,4], Daniela Muñoz[1,2], Mauricio Latorre[1,3,4,5]. [1]Centro UOH de Biología de Sistemas para la Sanidad Vegetal (BioSaV). Universidad de O'Higgins. San Fernando, Chile. [2]Laboratorio de Inmunidad Vegetal. Instituto de Ciencias Agroalimentarias, Animales y Ambientales. Campus Colchagua. Universidad de O'Higgins. San Fernando, Chile. [3]Centro de biología de sistemas para el estudio de comunidades extremófilas de relaves mineros (SYSTEMIX), Universidad de O'Higgins, Rancagua, Chile. [4]Laboratorio de Bioingeniería, Instituto de Ciencias de la Ingeniería, Universidad de O'Higgins, Rancagua, Chile. [5]Laboratorio de Bioinformática y Expresión Génica, INTA, Universidad de Chile, Santiago, Chile. [6]Laboratorio de Nematología. Instituto de Ciencias Agroalimentarias, Animales y Ambientales. Campus Colchagua. Universidad de O'Higgins. San Fernando, Chile. [7]Laboratorio de Ecología Microbiana y Biogeoquímica del Suelo. Instituto de Ciencias Agroalimentarias, Animales y Ambientales. Campus Colchagua. Universidad de O'Higgins. San Fernando, Chile.

Introduction: Systems biology, with its comprehensive approach, offers a thorough understanding of complex agricultural ecosystems by examining the interconnectedness of its components, including plants, soil, and microorganisms. This holistic perspective is essential for addressing multifaceted challenges such as pest and disease management in agronomic systems, which are influenced by a myriad of factors. A prime example is the study of nematode infestations in tomato crops. By applying systems biology principles, it's possible to identify key factors within the soil-microbiome-plant interaction that contribute to plant health or susceptibility to nematodes. This research aims to develop a model that captures the complex dynamics of these interactions, enabling the prediction of plant responses to different conditions and the identification of potential interventions to enhance crop resilience and productivity. Ultimately, this knowledge can inform the development of sustainable and effective agricultural practices, providing reassurance about the thoroughness of our research.
Materials and methods: The level of Meloidogyne spp. parasitism in tomato plants was determined by assessing plant damage, gall and egg counts, and disease index. Plant immune status was evaluated by quantifying the expression of PR1a, PR1b, Pti-5, Pi1, and Pi2 genes via RT-qPCR. Fruit productivity and quality, including sugar content and acidity,

were assessed. Soil from commercial farms with asymptomatic and symptomatic plants was analyzed for microbial community structure and activity. Soil enzyme activities (dehydrogenase, phosphatase, arylsulfatase, and urease) were measured, and the soil microbiota was characterized through 16S rRNA and ITS metabarcoding.
Results: Parasitism levels of Meloidogyne spp. were significantly higher in symptomatic tomato plants, correlating with reduced plant health and fruit quality. Immune gene expression analysis revealed compromised plant defenses. Nematode fauna communities differed between healthy and infested plants. However, a low difference was observed among enzymatic activities in the soils.
Discussion: The study revealed a strong association between Meloidogyne spp. infection and reduced plant health, characterized by decreased yield and fruit quality, and altered plant immunity. Currently, we are characterizing soil microbial communities, and identifying specific microbial taxa linked to nematode suppression will suggest potential avenues for developing sustainable nematode management strategies. The Center BioSaV will generate an integrated model to identify key underlying mechanisms of these interactions and will look for practical applications for crop protection.
Acknowledgment: Centro UOH de Biología de Sistemas para la Sanidad Vegetal. BioSaV-UOH. Universidad de O'Higgins.

Integrative and AI approaches to unravel the complexities of human diseases. Helder Nakaya[1,2] (hnakaya@usp.br). [1]Hospital Israelita Albert Einstein, São Paulo, Brazil; [2]Department of Clinical and Toxicological Analysis, School of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil.

Introduction: Human diseases are intricate systems arising from intricate interactions between genetic, environmental, and lifestyle factors. This study explores the application of integrative biology approaches to elucidate the underlying mechanisms of these complex pathologies.
Materials and methods: We integrate molecular biology and artificial intelligence techniques to generate insights into disease pathogenesis at the molecular level. Subsequently, we harness the power of advanced bioinformatics to analyze large-scale datasets, identify disease biomarkers, and predict therapeutic targets
Results: By combining these complementary approaches, we aim to foster a comprehensive understanding of human diseases, enabling the development of novel diagnostic tools and therapeutic interventions.
Discussion: Ultimately, this research contributes to the advancement of precision medicine and improved patient outcomes.
Acknowledgment: FAPESP 2018/14933-2

Symposium 10: From biomolecules, vesicles and nanoconstructs to biomedical applications.

Nanoformulations of curcumin and their applications in cancer prevention and treatment. Andrew F.G. Quest [1,2]. [1]Cellular Communication Laboratory, Center for Studies on Exercise, Metabolism and Cancer (CEMC), Institute of Biomedical Sciences (ICBM), Faculty of Medicine, University of Chile, Santiago, Chile. [2]Advanced Center for Chronic Diseases (ACCDiS), Faculty of Medicine, University of Chile, Santiago, Chile.

We have for many years focused on understanding how the scaffolding protein Caveolin-1 (CAV1) participates in cancer development and progression. In the initial years, these studies yielded solid evidence for the role of CAV1 as a tumor suppressor and insight to the mechanisms by which these effects are achieved. Perhaps, rather counter-intuitively, our follow-up studies uncovered how CAV1 can promote migration, invasion and metastasis. In the course of these initially in vitro studies in cells, we developed a mouse model that permitted corroborating specific aspects of our studies in vivo. Finally, our most recent research uncovered a role for CAV1 in the dissemination of systemic information as a key component in exosomes. Beyond the availability of advanced knowledge and resources for studies in the areas of biochemistry, molecular biology and cell biology, essential in these studies over the last 25 years here in Chile, was having access to an animal model that permitted demonstrating the ambiguity of CAV1 function in vivo. Of fundamental importance for the discussion here, is how this animal model then permitted evaluating the efficacy of specific inhibitors and nano-formulations of natural compounds in the treatment of cancer disease. In this context, I will focus attention on our studies with curcumin and how we obtained evidence for the efficacy of curcumin-containing nano-emulsions in the successful treatment not only of primary tumors but also metastatic disease, initially in a post-operatory mouse model and more recently in experiments evaluating the effects in dogs with a wide range of different types of tumors. In summary, these studies provide solid evidence supporting the notion that research efforts leading to advances in the understanding of basic mechanisms in biochemistry, cell and molecular biology can lead to therapeutic applications for the treatment of disease.

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Combining tradition with upcoming technologies: novel sources of extracellular vesicles for wound healing applications. Gabriela Zavala[1], Simon Alvarez[1], Pamina Contreras[1], Florencia Pomareda[1], Belen Olivares[2], Veronica Silva[1], Sebastian Aguayo[3,4], Christina Schuh[1]. [1]Centro de Medicina Regenerativa, Facultad de Medicina, Clínica Alemana-Universidad del Desarrollo, Santiago, Chile. [2]Centro de Química Medica, Facultad de Medicina, Clínica Alemana-Universidad del Desarrollo, Santiago, Chile. [3] Dentistry School, Faculty of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile. [4]Institute for Biological and Medical Engineering, Schools of Engineering, Medicine and Biological Sciences, Pontificia Universidad Católica de Chile.

Introduction: It is estimated that by 2030, approximately 7 million people will be living with non-healing wounds, which displays a significant burden for both, patients and healthcare systems. Today, healing success strongly depends on the medical attention given (e.g. debridement, antibiotic treatments, etc) and there are few "all-in-one" approaches available. Traditional medicine offers a number of versatile candidates for wound healing. However, plant extracts have been shown to be difficult to standardize. Over the last decades, extracellular vesicles (EVs) have become increasingly popular. EVs are capable of interkingdom communication, offering a wide range of therapeutic applications. We assessed the potential of isolated EVs from known natural products *in vitro* and *in vivo* to design novel treatments for complex wounds.
Methods: EVs from different sources (e.g. Royal Jelly, Aloe vera) were isolated and characterized with Nanoparticle Tracking Analysis, Transmission Electron Microscopy, as well as proteomics. Anti-inflammatory effects of EVs were evaluated with ELISA and RAW 264.7 macrophages after LPS stimulation. Antibacterial potential was assessed with Staphylococcus aureus 25923 in a microplate assay. Anti-fibrotic effects were tested in collagen-contraction assays. Effects on scarring and wound healing were evaluated after 12 days in a splinted wound model (planimetry, histology, ELISA of pro-inflammatory cytokines).
Results: EVs were isolated in a reproducible manner from honeybee products and plants, displaying a particle size of less than 150nm. Proteomics revealed presence of active proteins, giving insight into the mechanisms of action. All tested candidate EVs significantly decreased pro-inflammatory cytokines. However, only honeybee EVs were antibacterial. In functional *in vivo* experiments, EVs accelerated regeneration, decreased inflammation and improved cellular composition in the wound bed.
Discussion: Our studies demonstrate that plants and honeybee products are readily available sources for therapeutic EVs. By isolating EVs from the raw material, the known therapeutic effects are maintained while potential contaminants are removed, making them attractive candidates for wound healing.
Acknowledgments: This work was supported by ANID FONDEF IdEA ID22I10099 and Fondecyt 1220803.

Secretome derived from mesenchymal stem cells as a new biodrug for the treatment of drug addictions: Toward the identification of therapeutic molecules. Fernando Ezquer[a,b] (eezquer@udd.cl); Javiera Gallardo[a,b]; David Ramírez[b,c,]; Ignacio Valenzuela Martínez[c]; Nicolás Medina[c]; María Elena Quintanilla[d]; Paola Morales[b,d]; Marcelo Ezquer[a]; Yedy Israel[a,b,e]. [a]Center for Regenerative Medicine, School of Medicine, Clínica Alemana-Universidad del Desarrollo, Santiago, Chile. [b]Research Center for the Development of Novel Therapeutic Alternatives for Alcohol Use Disorders, Santiago, Chile. [d]Departamento de Farmacología, Facultad de Ciencias Biomédicas, Universidad de Concepción, Concepción, Chile. [e]Molecular and Clinical Pharmacology Program, School of Medicine, Universidad de Chile, Santiago, Chile.

Chronic consumption of addictive drugs leads to neuroinflammation and oxidative stress, which inhibit the astrocyte Na-glutamate-transporter (GLT-1) in the brain reward circuit, proposed to perpetuate drug addiction. The secretome derived from mesenchymal stem cells (MSCs) contains potent anti-inflammatory and antioxidant molecules. We demonstrated that the intranasal administration of the secretome derived human MSCs to rats voluntarily drinking ethanol, nicotine or morphine: (a) inhibited voluntary drug intake and relapse; (b) inhibited drug-induced neuroinflammation and oxidative stress; and (c) increased brain GLT-1 levels.
Knockdown of GLT-1, by the administration of an antisense oligonucleotide, fully abolished the inhibitory effect of MSC-derived secretome on drug intake, suggesting that GLT-1 mediates the therapeutic effects of MSCs. Using proteomic analysis and artificial intelligence, we conducted multiple protein-protein interaction analysis in the GLT-1 network (PPI-analysis), which allowed the identification of proteins in the MSC secretome that are strongly represented in the GLT-1-PPIs network. The most promising candidates were recombinantly produced and encapsulated in cationic liposomes for intranasal testing in animal models of drug addiction.
Overall, these studies indicate that the administration of MSC-derived secretome or specific molecules contained within it offers translational opportunities for the treatment of drug use disorders.
Supported by: ANID #ACT210012 and FONDECYT #1240162 grants to FE

Strategies for finding biomarkers to detect and combat radioresistance. Ina Kurth. German Cancer Research Center (DKFZ), Division of Radiooncology/ Radiobiology, Heidelberg, Germany. German Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ), Heidelberg, Germany. National Center for Tumor Diseases (NCT), Partner Site Dresden, Germany: German Cancer Research Center (DKFZ), Heidelberg, Germany, Faculty of Medicine and University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany, and Helmholtz Association / Helmholtz-Zentrum Dresden - Rossendorf (HZDR), Dresden, Germany.

The development of patient-specific, individualized treatment strategies is one of the main goals of modern clinical radiation oncology. Already today, radiotherapy is highly individualized in terms of dose distribution based on anatomical and clinical information. Technological improvements and new biological concepts will drive the evolution of radiation oncology to further expand the therapeutic scope and precision of radiotherapy. These include particle therapy, advanced image guidance, and treatment adaptation and therapeutic intervention during therapy, e.g., based on novel biological concepts to be discovered. Tumor radioresistance often impedes the success of radiotherapy. Due to high tumor heterogeneity, not every patient will likely benefit from the current state-of-the-art therapy against a certain tumor type. Measurable imaging parameters e.g. micromillieu, metabolism, tumor biomarker via positron emission tomography or magnetic resonance (PET or MR) improve the concept of personalization and precision in medicine by facilitating patient stratification, risk assessment and adaptation during therapy. Biomarkers constitute the basis for further individualization of the treatment. High molecular throughput assays and bio-imaging technologies have led to a rapid emergence of novel biomarkers.
The combination of omics (genomics, proteomics) with image data further fosters the personalization and precision of cancer medicine. Sound knowledge of the biological radioresistance is essential to understand mechanisms leading to i.e. relapse after therapy. The talk will provide insight into some of the strategies we and others in the field are using to discover novel translatable biomarkers.

Symposium 11: Genomic surveillance of microorganisms.

Genomic Epidemiology of Emergent Infectious Diseases, GENE2DIS. Juan A. Ugalde[1], Cecilia Vial[2], Rafael Araos[2], Cecilia Poli[2,3], Pablo Vial[2,3], Katia Abarca[4], Marcela Ferres[4], Johanna Acevedo[2], Thomas Weitzel[2,3]. [1]Centro de Bioinformatica y Biología Integrativa, Facultad de Ciencias de la Vida, Universidad Andres Bello. Republica 440, Santiago, Chile. [2]Instituto de Ciencias e Innovación en Medicina, Facultad de Medicina, Clinica Alemana Universidad del Desarrollo, Av. Plaza 680, Santiago, Chile. [3]Clinica Alemana, Vitacura 5951, Santiago, Chile. [4]Departamento de Enfermedades Infecciosas, Facultad de Medicina, Universidad Católica de Chile, Marcoleta 340, Santiago, Chile.

Surveillance of emerging infectious diseases is fundamental for the establishment of rapid responses, such as medical care, and also for understanding their epidemiology. These diseases are commonly caused by existing pathogens that expand their host range and/or by the emergence of novel, previously undetected pathogens. An example of this was the emergence of the SARS-CoV-2 virus, which caused a pandemic with consequences that vividly illustrate the effects of a rapidly evolving virus requiring a swift response.
To quickly respond to these requirements, we need novel multidisciplinary approaches, including teams from diverse areas, such as molecular biology, bioinformatics,

epidemiology, and infectious diseases, to develop novel strategies and platforms for the surveillance of these emerging infectious diseases. Likewise, collaboration between academic and governmental institutions is crucial to the success and rapid dissemination of findings.
The Gene2DIS project, funded by ANID, is a collaborative project that seeks to fill these gaps in genomic epidemiology through research on emerging pathogens and developing surveillance platforms based on genomic strategies. In this talk, we will show some of our results up to date, which include work on the genomic epidemiology of SARS-CoV-2 and Hantavirus (ANDV), genomics of microbial pathogens such as Rickettsiales and *Streptococcus pyogenes*, and the use of metagenomics for the study of infections of unknown origin.
Funding: Anillo ATE 220061, Fondecyt 1241530, Fondecyt 1221209

Situación de VIH en Chile. Fernando Valiente-Echeverría; Claudia P. Cortés; Ricardo Soto-Rifo. Laboratorio de Virología Molecular y Celular, Programa de Virología, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Santiago, Chile.

A más de 40 años del reconocimiento de los primeros casos, la pandemia del virus de la inmunodeficiencia humana y el síndrome de inmunodeficiencia adquirida (VIH/SIDA) aún representa un importante problema de salud pública con un promedio de 1,3 millones de nuevas infecciones al año. Desde el comienzo de la pandemia a principios de los años 80, más de 80 millones de personas se han infectado con el VIH y 40 millones han muerto por una enfermedad relacionada con el SIDA. Según el último informe de ONUSIDA, actualmente hay 39 millones de personas que viven con el VIH en todo el mundo, de las cuales 28 millones tienen acceso a la terapia antirretroviral.
Durante los últimos años, Chile ha experimentado un aumento importante en la prevalencia de infecciones por VIH alcanzando las 84.000 personas al 2021 y con cinco mil nuevas infecciones reportadas durante el mismo año. Al 30 de junio del 2024, el ISP reporta 2244 casos por lo que, al igual que en 2023, se prevén cifras cercanas a los 5 mil nuevos casos para el 20234 Si bien en Chile la terapia antirretroviral (TAR) debe ser brindada por ley y la gran mayoría de los diagnosticados se encuentran bajo tratamiento, un 31% de los nuevos casos de VIH se detectan en etapas avanzadas de la infección (etapa SIDA). Desafortunadamente, se predice que las nuevas infecciones continuarán aumentando en Chile y, pese a que la infección por VIH/SIDA es una amenaza importante para nuestra sociedad y economía, existen escasos grupos de investigación multidisciplinarios abocados a estudiar los diversos aspectos de esta pandemia. En América Latina, este tipo de centros sólo existe en Argentina, Brasil, Perú y México.
Ante esta necesidad, generamos un grupo de trabajo transversal en VIH/SIDA en la Facultad de Medicina de la Universidad de Chile liderado por investigadores con diversas experticias y un objetivo común: contribuir a la lucha mundial en contra de la pandemia del VIH/SIDA, generando evidencias y transfiriendo el conocimiento con el fin de generar nueva

evidencia y mejorar la calidad de vida de las personas que viven con el virus mientras situamos a Chile como un líder Regional en la investigación integral en VIH/SIDA.
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Detection and incidence of respiratory viruses in the school environment and their impact on the nasopharyngeal microbiota of children -Anillo School-Microbe. Jorge Valdes[3], Nicolás Pacheco Camus[1], Aldo Gaggero Brillouet[2], Gabriel Krüger[1], Francisca Urbina Arce[1], Francisco Remonsellez Fuentes[4,5], Juan Castro-Severyn[1,4,5], Gloria Arriagada[6], Fernando Valiente[7], Claudia Saavedra Sanchez[1]. [1]Laboratorio de Microbiología Molecular, Facultad de Ciencias de la Vida, Universidad Andres Bello,Santiago, Chile. [2]Laboratorio de Virologia Ambiental. Programa de Virología. ICBM. Facultad de Medicina. Universidad de Chile. [3]Center for Bioinformatics and Integrative Biology, Facultad de Ciencias de la Vida, Universidad, Andres Bello, Santiago, Chile. [4]Laboratorio de Microbiología Aplicada y Extremófilos, Departamento de Ingeniería Química, Universidad Católica del Norte, Antofagasta, Chile. [5]Centro de Investigación Tecnológica del Agua en el Desierto (CEITSAZA), Universidad Católica del Norte, Antofagasta, Chile. [6]Instituto de Ciencias Biomedicas, Facultad de Medicina y Facultad de Ciencias de la Vida, Universidad Andres Bello, Santiago 8370071, Chile. [7]Molecular and Cellular Virology Laboratory, Virology Program, Institute of Biomedical Sciences, Faculty of Medicine, Universidad de Chile, Santiago, Chile.

The study of the nasopharyngeal microbiota of children and its interaction with respiratory viruses has gained relevance, given that the microbial composition of the upper respiratory tract influences health and susceptibility to infections. The microbiota acts as a protective barrier, modulating the immune response and limiting the colonization of pathogens. However, viral infections such as those caused by the influenza virus and respiratory syncytial virus (RSV) alter this microbiota, increasing the risk of secondary infections.
The Anillo School-Microbe project aims to analyze the presence of respiratory viruses in school environments and their impact on the nasopharyngeal microbiota of children. In the first phase of the study, nasopharyngeal and air samples were collected during the weeks of high incidence of respiratory diseases (Epidemiological Week 24-25). Using molecular techniques, viruses such as influenza, RSV, seasonal coronaviruses and others were identified, revealing a high prevalence of these pathogens in schools, suggesting that these environments are important foci of viral transmission. In the second phase, metataxonomic analysis using 16S rRNA gene sequencing allowed the assessment of the effects of viral infections on microbiota diversity and composition. Alpha and beta diversity analyses revealed significant changes in the microbiota during infections, with seasonal variations affecting key bacteria such as Lactobacillus, Bifidobacterium, Moraxella and Haemophilus. Hierarchical clustering of the microbiomes identified four main groups, while network analysis highlighted competitive and synergistic interactions between taxa. These

bioinformatic findings underline the influence of viral infections on the stability of the childhood microbiome, affecting susceptibility to future infections. The results suggest the need to implement prevention strategies in school environments and develop interventions that mitigate the negative effects of viral infections on the microbiota.

El virus dengue en el Peru y las americas, 2023. García Mendoza María Paquita. Responsable del Laboratorio de Referencia Nacional de Metaxénicas y Zoonosis Virales – Instituto Nacional de Salud – Perú.

Introducción: El dengue representa un desafío creciente de salud pública en Perú y las Américas, con ciclos epidémicos cada 3 a 5 años 1,2,3. Los cuatro serotipos del virus (DENV-1, DENV-2, DENV-3 y DENV-4) han circulado desde 1990, y la propagación del vector Aedes aegypti ha exacerbado su incidencia, especialmente tras la disminución en actividades de control durante la pandemia de COVID-19. El aumento de la incidencia del dengue y la gravedad de la enfermedad puede estar asociada al cambio de los genotipos circulantes, así como a la evolución viral 1,4. En el 2019, se identificó en Perú un nuevo genotipo del serotipo DENV-2, el Cosmopolitan, tras un brote significativo en Madre de Dios[5]. Este hallazgo subrayó la necesidad de fortalecer la vigilancia genómica para evaluar la dispersión de nuevos genotipos y su impacto en la salud pública, por lo que se secuenciaron virus de dengue procedentes de diferentes regiones del Perú durante el año 2023.
Metodología: Se seleccionaron 55 muestras de pacientes y 18 muestras de fallecidos diagnosticados con dengue mediante RT-PCR, recolectadas entre marzo y agosto del 2023, procedentes de las diferentes regiones endémicas del Perú, con el propósito de realizar un análisis genómico detallado.
Los productos amplificados fueron procesados usando el Kit COVIDSeq-Ruo (Illumina) mediante secuenciación genómica de nueva generación (NGS), para luego cargarlos en el secuenciador genómico Miniseq (Illumina). Los genomas de las bases de datos y los secuenciados fueron alineados con el programa MAFFT. Estas fueron utilizadas para un análisis filogenético con la metodología de Maximum Likelihood (ML), para cada set de datos, con el programa IQTREE2 calculando el valor bootstrap.
Resultados: Se caracterizaron 55 genomas: 13 (23.6%) de DENV-1; y 39 (79.9%) de DENV-2 y 3 DEN3 (5,45%). Las muestras de DENV-1 corresponden al genotipo V, este genotipo circula desde los años 70s en el Caribe y las Américas. Las muestras de DENV-2 corresponden al genotipo Cosmopolitan, este genotipo fue detectado por primera vez en el Perú en 2019 (Madre de Dios)[5] y se ha dispersado a las diferentes regiones de Perú. Las muestras de DENV-3 corresponden al genotipo III, solo ha sido caracterizado en muestras de Lima metropolitana. Se reportan las mutaciones E:K343R y E:S338L para el serotipo DENV-1 en la región de la envoltura (Envelope, E). Se reportan las mutaciones E:L458F, E:I129V, E:I126T y E:V309A, E:V484I para el serotipo DENV-2 en la región de la envoltura (Envelope, E).

Las muestras con DENV-1 de fallecidos corresponden al genotipo V, este genotipo circula desde los años 70s en el Caribe y las Américas. En Sudamérica especialmente el Perú está presente desde 1995. Se reportan las mutaciones visualizadas en 11 muestras tales como NS1: M178I clado I y clado III y NS4B:A20T clado I.
Las muestras con DENV-2 de fallecidos corresponden al genotipo Cosmopolitan, este genotipo fue detectado por primera vez en el Perú en 2019 (Madre de Dios) y se ha dispersado a las diferentes regiones de Perú y los algunos países de las Américas. Se reporta mutaciones visualizadas 7 muestras tales como NS5: E282 clado I ; M: A69S, NS5: A637T lado II y NS2A: I33T.
Conclusiones: Se identificaron y caracterizaron genomas de DENV-1, DENV-2 y DEN3 en muestras de pacientes, así como también en muestras de fallecidos en los brotes de Perú durante el 2023, Así como también destacando la presencia de mutaciones que podrían influir en la virulencia y propagación del virus.

Symposium 12: Discovering new generation proteomics, a tool applied from life of science to clinical application.

Unraveling the Links Between Host and Microbiome in Health and Disease with Ultra-Sensitive Metaproteomics. David Gomez-Varela. Director of the University of Vienna/Bruker Center of Excellence for Metaproteomics. Division of Pharmacology & Toxicology, Department of Pharmaceutical Sciences, Faculty of Life Sciences, University of Vienna, Austria.

Functional interplays between members of microbial communities (microbiomes) are crucial for the health of all planetary ecosystems. Metaproteomics, with its ability to unveil unique insights into microbial function and interaction with host environments, presents a promising tool for microbiome research. However, the inherent complexity of these communities poses major challenges in terms of sensitivity, depth, and reproducibility of protein quantification, especially for low-abundance microorganisms. Novel technological advancements are crucial to unlocking the full potential of metaproteomics for thorough investigation of microbiological habitats.
The latest generation of highly sensitive mass spectrometers, coupled with optimized LC chromatographic columns and ionization sources, achieves astounding depth, sensitivity, and quantitative performance in single-organism samples, ranging from thousands to individual cells. However, their efficacy in metaproteomic samples remains unexplored. I will present the benefits of combining the new Tims TOF Ultra mass spectrometer with an optimized ionization source and an Ultra High-Performance Liquid Chromatography column. Using mouse feces as a model system, we demonstrate a drastic improvement in the number of identified and quantified protein groups and associated functional pathways for both the microbiome and the host (exceeding 40,000 groups, 220 KEGG and GO terms).

Additionally, we show a significant increase in the number of bacterial species quantifiable with high reproducibility (more than 200), even from minute sample amounts (1 ng of peptides) and rapid analysis (30-minute chromatographic gradients). The advantages of this new state-of-the-art for revealing novel mechanisms of disease in mouse preclinical models will be presented. Finally, I will show our efforts to push the sensitivity limits by successfully detecting species-specific peptides at a single-bacterium-resolution level (500 femtograms). These results showcase the current potential of ultra-sensitive metaproteomics in various biotechnological fields (e.g., large-scale clinical metaproteomic studies) and pave the way for new frontiers in unexplored biological territories (e.g., single-bacterium-proteomics).

The story of discovering the functions of RUBCNL/PACER – a protein at the interface between pathways.

Ute Woehlbier[1], Leonardo Rodriguez[1], Luis Labrador1, Sebastian Beltran[1], Cristian Bergmann[1], Fernanda Hernandez[1], Francisco Muñoz-Carvajal[2], Diego Rojas-Rivera[2], Melissa Nassif[2], Bredford Kerr[3], Mathieu Bertrand[4], Patricio Manque[1,5]. [1]Center for Integrative Biology (CIB), Faculty of Science, Universidad Mayor, Chile. [2]Center for Biomedicine, Universidad Mayor, Chile. [3]Centro de Biología Celular y Biomedicina (CEBICEM), Universidad San Sebastián, Chile. [4]Department of Biomedical Molecular Biology, Ghent University, Belgium. [5]Centro de Oncologia de Precision (COP), Universidad Mayor, Chile.

The protein PACER (also called RUBCNL) was identified to be involved in the pathogenesis of Amyotrophic lateral sclerosis (ALS) using a bioinformatic approach to predict novel candidates involved in the disease. PACER levels are diminished in post-mortem spinal cord tissue from ALS patients. In ALS mouse models we found a similar depletion of PACER protein levels in both pre-symptomatic and symptomatic stages. Furthermore, in the CNS PACER is mainly expressed in neurons, including motor neurons in the spinal cord. PACER loss of function in cellular models results in reduced autophagy activity, augmented SOD1 aggregation, and sensitization of motor neurons to cell death. In another cellular context, we recently showed that PACER has dual functions in autophagy and cell death pathways. Interestingly, PACER represses inflammatory cytokine TNF induced toxicity by negatively regulating RIPK1 kinase-dependent apoptosis and necroptosis, a function found to be independent of its parallel role in autophagy. Using diverse strategies we study how the modulation of PACER affects these pathways and can be targeted for disease intervention.

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Biofluid proteomics study a snapshot of human health. Hernandez Mauricio[1], Saldivia Pablo[1,2], Nourdin Guillermo[1,2], Castro Felipe[1,2], Antilef Barbara[1,2], Hernandez Sergio[1], Vargas Cristian[1], Koch Elard[1,2]. [1]Biotechnology Division of MELISA institute, San Pedro de la Paz, Chile. [2]Fundación de Investigación San Ramon (FISAR).

Biofluids have assumed a significant role in the clinical proteomic study of large cohorts in recent years, primarily due to their ease of collection and storage. However, each type of fluid requires specific preparation and analysis techniques from a proteomic standpoint. For instance, obtaining highly detailed results in plasma is particularly challenging due to the polarized dynamic range of proteins. The 20 most abundant proteins in plasma account for more than 98% of total protein content. Additionally, these abundant proteins are present in mg/mL concentrations, while the less abundant proteins are found in fg/mL concentrations, which greatly complicates their analysis. To address this, depletion strategies have been developed to remove these abundant proteins, thereby significantly increasing the depth and number of detectable markers. Furthermore, advancements in mass spectrometry have enabled high-throughput analysis of samples, making it feasible to analyze cohorts that, until recently, were unmanageable due to time constraints and associated costs. In our laboratory, we have developed a range of methodologies for advanced proteomic analysis in biofluids, with a particular focus on cohort studies. Using these methodologies, we have successfully analyzed fluids such as plasma, urine, saliva, and cervicovaginal fluid, yielding excellent results across various clinical contexts. These include studies on female reproductive health through the analysis of cervicovaginal fluid, and investigations into the long-term effects of COVID-19 through serum analysis.
Acknowledgement: FISAR Grant Collaborative founding 012024.

Mito Stress: A potential mechanism for T cell exhaustion. Dr. Estefanía Nova Lamperti. Departamento de Bioquímica Clínica e Inmunología, Facultad de Farmacia, Universidad de Concepción.

Oral squamous cell carcinoma (OSCC) is the most frequent type of oral cancer, and it has been reported that the OSCC tumour microenvironment (TME) induces impaired T cell responses, promoting an exhausted phenotype and metabolic reprogramming. The mitochondria are the main metabolic organelle and in recent years it has been shown that several cells have the capacity to transfer mitochondria, including cancer cells. However, up to date, it has not been evaluated whether mitochondria transfer from cancer cells to T lymphocytes promotes an exhausted phenotype in T helper cells. In this study, we evaluated the functional and metabolic effect of artificial mitochondria transfer from the oral cancer cells HSC-3 into activated CD4+ T cells. Mitochondria from oral cancer cell were labelled with MitoTracker dye, isolated and mitocepted into activated $CD4^+$ T cells. Then, cell proliferation, expression of surface molecules and cytokine secretion after mitochondria transfer were analyzed by flow cytometry to evaluate T cell exhaustion. In addition, the

metabolome and the proteome in CD4+ T cells were analyzed after mitochondrial transfer by mass spectrometry. Our results showed that mitocepted $CD4^+$ T cells significantly decrease proliferation and increase the expression of TIGIT, CTLA-4, PD-1, PDL-1 y LAG-3 in comparison to the control group. Regarding cytokine secretion, a significant decrease was observed in the mitocepted group for IFN-gamma, TNF-alpha, IL-10, and IL-4 production, but not for IL-2 or IL-17. Metabolomic and proteomic pathways revealed that T cells that acquired malignant mitochondrial showed a hypoxic state with more reactive oxidative species (ROS) production, more glycolysis and reduction in the pyruvate dehydrogenase cofactor Vitamin B1. Finally, we confirmed that mitocepted CD4+ T cells increased glucose uptake, glucose consumption and lactate and ROS production. In summary, the acquisition of isolated mitochondria from HSC-3 cancer cells by $CD4^+$ T lymphocyte induces recipient mitochondrial oxidative stress, and a possible reduction of the Krebs cycle mediated by insufficient Vitamin B1, forcing the cell to use glycolysis as a salvage pathway. This effect impairs the antitumor response by promoting exhausted and dysfunctional $CD4^+$ T cells.

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ABSTRACTS NEW MEMBERS SESSION

OPA1 and disease-causing mutants perturb mitochondrial nucleoid distribution. Macuada J.[1], Molina-Riquelme I.[1], Vidal G.[2,3], Pérez-Bravo N.[1], Vásquez-Trincado C.[1], Aedo G.[1,2], Lagos D.[1,4], Horvath R.[4], Rudge T.J.[2,3,5], Cartes-Saavedra B.[1,6], Eisner V.[1](veisner@bio.puc.cl). [1]Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile. [2]Institute for Biological and Medical Engineering, Schools of Engineering, Biology, and Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile. [3]Interdisciplinary Computing and Complex Biosystems (ICOS) research group, School of Computing, Newcastle University, Newcastle upon Tyne, UK. [4]Department of Clinical Neurosciences, John Van Geest Centre for Brain Repair, University of Cambridge, Ed Adrian Building, Robinson Way, Cambridge, CB2 0PY, UK. [5]Department of Chemical and Bioprocess Engineering, School of Engineering, Pontificia Universidad Católica de Chile, Santiago, Chile. [6]MitoCare Center for Mitochondrial Imaging Research and Diagnostics, Department of Pathology and Genomic Medicine, Thomas Jefferson University, Philadelphia, PA, USA.

Introduction: Optic atrophy protein 1 (OPA1) mediates inner mitochondrial membrane (IMM) fusion and cristae organization. Mutations in OPA1 cause autosomal dominant optic atrophy (ADOA), a leading cause of blindness. Cells from ADOA patients show impaired mitochondrial fusion, cristae structure, bioenergetic function, and mitochondrial DNA (mtDNA) integrity. The mtDNA encodes electron transport chain subunits and is packaged into nucleoids spread within the mitochondrial population. Nucleoids interact with the IMM, and their distribution is tightly linked to mitochondrial fusion and cristae shaping. Yet, little is known about the physio-pathological relevance of nucleoid distribution.
Materials and Methods: We studied the effect of OPA1 and ADOA-associated mutants on nucleoid distribution using high-resolution confocal microscopy. We applied a novel model incorporating the mitochondrial context, separating nucleoid distribution into the array in the mitochondrial population and intramitochondrial longitudinal distribution.
Results: Opa1-null cells showed decreased mtDNA levels and nucleoid abundance. Also, loss of Opa1 led to an altered distribution of nucleoids in the mitochondrial population, loss of cristae periodicity, and altered nucleoids to cristae proximity partly rescued by OPA1 isoform 1. Overexpression of WT OPA1 or ADOA-causing mutants c.870+5G>A or c.2713C>T in WT cells, showed perturbed nucleoid array in the mitochondria population associated with cristae disorganization. Opa1-null and cells overexpressing ADOA mutants accumulated mitochondria without nucleoids. Interestingly, intramitochondrial nucleoid distribution was only altered in Opa1-null cells.
Discussion: Altogether, our results highlight the relevance of OPA1 in nucleoid distribution in the mitochondrial landscape and at a single-organelle level and shed light on new components of ADOA etiology.
Acknowledgment: Fondecyt 1231557

Alternative splicing as a key regulator of root responses to salinity in Arabidopsis. O'Brien José A. (jobrieno@uc.cl). School of Biological Sciences and School of Agronomy and Natural Systems, Pontificia Universidad Católica de Chile, Santiago, Chile.

Introduction: Alternative splicing (AS) is a crucial mechanism in eukaryotic organisms for both post-transcriptional regulation of gene expression and the diversification of the genome. In plants, it plays a significant role in the response to different abiotic stresses, including salt stress. However, the extent of differential AS regulation and its role in response to salt stress treatments has not been fully explored so far.
Materials and Methods: Here we studied the role of AS in *Arabidopsis thaliana* in response to salt stress using a genomic approach. We perform an mRNA-seq experiment at 2, 4 and 24h of NaCl treatment using both short-reads (Illumina) and long-reads (PacBio) technologies.
Results: To study the role of AS in Arabidopsis root development under salt stress we used the pre-mRNA splicing inhibitor Herboxidiene (HEX). This was evaluated in Col-0 and plant hormone reporter lines. Furthermore, we performed a comprehensive analysis of AS regulation in response to salt stress using publicly available short-read transcriptomic data from 30 different studies of Arabidopsis. Moreover, our results from mRNA-seq shows that salt stress responses depend, at least in part, from pre-mRNA splicing. Altering this process has an impact in hormone signalling in roots. Interestingly, in response to salt stress, the intersection between differentially expressed genes (DEGs) and differential AS genes (DAS) is relatively small, indicating that AS acts as an independent regulatory layer in response to salt stress. Also, the intersection of AS-regulated genes between different organs is small, indicating that distinct sets of genes are regulated by AS under salt stress in different organs. Finally, we identified a significant proportion of AS events that are potentially unique to salt stress.
Discussion: Overall, our study sheds light on the complex landscape of AS regulation in response to salinity and highlights the importance of AS regulatory mechanisms.
Acknowledgment: FONDECYT 1221832

Exploring the Bioactive Potential of Disulfide-rich Peptides from Chilean Native Plants. Fabiola Morales[1], Paula Santana[2], Nathalia Dias[3], Cristian Sillagana[4]), Paola G.Ojeda[4](paola.ojeda@uss.cl). [1]Universidad Católica del Maule, Maule, Chile. [2]Universidad Autónoma de Chile, Santiago, Chile, [3]Universidad de la Frontera, Temuco, Chile. [4]Escuela de Química y Farmacia, Facultad de Medicina y Ciencia, Universidad San Sebastián, Valdivia, Chile.

Introduction: Disulfide-rich peptides (DRPs) from Chilean native plants present unique opportunities for antimicrobial and insecticidal applications due to their structural stability and potent bioactivities. These peptides are being explored as novel agents to combat drug-

resistant bacteria and agricultural pests. This study focuses on the extraction, purification, and application of DRPs from Chilean flora, assessing their bioactive potential and practical use. **Materials and Methods:** Peptides were extracted from selected Chilean native plants using crude extraction methods followed by solid-phase extraction (SPE) fractionation. The antimicrobial activity of these extracts was tested against *Listeria monocytogenes, Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureu*s. Insecticidal efficacy was evaluated through bioassays on common agricultural pests. Peptide compositions were analyzed using mass spectrometry. **Results:** The DRPs demonstrated antimicrobial effects against *Listeria monocytogenes and Staphylococcus aureus*, with no activity observed against gram-negative strains like *Escherichia coli and Pseudomonas aeruginosa.* However, no significant insecticidal properties were observed in larvae bioassays.
Discussion: These findings highlight the potential of DRPs from Chilean native plants as potential antimicrobial and insecticidal agents. The study suggests that these peptides could be developed into valuable biotechnological tools for addressing challenges in both healthcare and agriculture. However, further characterization of the fractions needs to be conducted to optimize their efficacy and minimize toxicity.
Acknowledgment: This work was supported by Fondecyt 11220738

CRISPR-Cas-based diagnostics for terrestrial biosecurity using convolutional neural networks for streamlined detection of closely related invasive rodent species.
Benjamín Durán-Vinet[1] (benjamin.duran-vinet@postgrad.otago.ac.nz), Daniel R. Taylor[2], Anna Clark[1], Antoinette J. Piaggio[2], Neil Gemmell[1]. [1]Department of Anatomy, School of Biomedical Sciences, University of Otago, Dunedin, 9016, New Zealand. [2] USDA APHIS WS National Wildlife Research Center, Fort Collins, Colorado, United States.

Introduction: Terrestrial ecosystems face escalating challenges due to climate change and anthropogenic activities, broadly impacting ecosystem sustainability and related economic activities. The cost of invasive rodents alone exceeds $19 billion annually in the United States, with global estimates likely being much higher considering all affected sectors and regions. Therefore, early detection of invasive rodent populations is crucial for effective control and eradication efforts. Environmental DNA (eDNA) offers an easy, minimally invasive method to survey the presence of invasive species from water sources. Additionally, CRISPR-Cas technology provides a novel, reliable, and sensitive diagnostic platform that can be programmed through RNA sequences known as CRISPR RNAs (crRNAs).
Material and Methods: Our work proposes an innovative approach combining a convolutional neural network termed ADAPT (Activity-informed Design with All-inclusive Patrolling of Targets) that is trained to optimise CRISPR-Cas13-based diagnostics. ADAPT identifies optimal crRNA configurations to maximize diagnostic activity while minimizing off-

target activity. We applied the resulting crRNAs of this pipeline to detect *Rattus rattus* (Black rat) and *R. exulans* (Polynesian rat) in synthetic and genomic DNA samples.
Results: Preliminary *in vitro* diagnostics fluorescence results showcase significant fold changes (>10) in synthetic DNA samples in attomolar ranges and genomic DNA samples within one hour of reaction time and negligible off-target activity. These findings highlight the potential of artificial intelligence to streamline the deployment of CRISPR-Cas-based methods as an environmental biosecurity technology capable of providing quick and reliable results to complement pest control and eradication efforts. Furthermore, similar eDNA approaches could be used for disease detection and the ecological management of endangered species.
Funding acknowledgement: Financial support was provided by the US Air Force Pacific Air Forces and the 611th Civil Engineer Squadron through the Wake Atoll Rat Eradication Project. The funders had no role in study design, data collection nor analysis. This research was also supported in part by the U.S. Department of Agriculture, Animal Plant Inspection Service, Wildlife Services, National Wildlife Research Center. The findings and conclusions in this publication are those of the author(s) and should not be construed to represent any official USDA or U.S. Government determination or policy.

Translational mechanisms during stress in *Saccharomyces cerevisiae*. From academia to industry
Clara A. Solari (draclarasolari@gmail.com). ByBug

The regulation of gene expression is a complex process involving multiple layers of control, both at the transcriptional and translational levels. Throughout my research, I have studied various mechanisms of translational control, including differential mRNA localization, cytoplasmic granules, and the role of Protein Kinase A (PKA) in translation regulation in *Saccharomyces cerevisiae*. In my doctoral research, I explored the riboproteome during the exit from quiescence in yeast, providing insights into how cells reprogram their translation machinery in response to changing environmental conditions. Specifically, my work highlights the remodeling of the riboproteome as a crucial mechanism for modulating translation during the transition from a quiescent to an proliferative state. During my new member presentation, I will discuss how all these factors collectively contribute to the fine-tuning of protein synthesis, particularly under stress conditions, and are essential for cellular adaptation and survival. In addition to my academic research, I will share my experience as a scientist in industry. First, at Abalone Bio with a screening platform for functional therapeutic antibodies in yeast. And currently, at ByBug, a chilean biotech company which developed an innovative platform for the expression of recombinant proteins in larvae of *Hermetia illucens*, black soldier fly. This work represents a novel application of our understanding of gene expression regulation in a practical and industrial context, offering new opportunities for sustainable biotechnology.

Not All Spliceosomes Are Created Equal: RNA Modifications and snRNA Variants as Sources of Spliceosome Heterogeneity in Cellular Differentiation and Cancer. Méndez C[1,2], Sepúlveda T[1], Squicciarini V[1], Adrianzen R[1], Munita R[1].(roberto.munita@ciq.uchile.cl). [1]Advanced Center for Chronic Diseases (ACCDiS), Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmaceuticas, Universidad de Chile, Santiago, Chile. [2]Departamento de Bioinformática, Facultad de Ingeniería, Universidad de Talca, Chile.

Introduction: The spliceosome is a complex ribonucleoprotein machinery comprised of five small nuclear RNAs (snRNAs), U1, U2, U4, U5, and U6, and over 150 interacting proteins. These snRNAs are transcribed from multiple gene families in the human genome, giving rise to diverse snRNA variants with specific sequence alterations. Moreover, the snRNAs undergo post-transcriptional modifications, such as 2'-O-methylation crucial for spliceosome function. Intriguingly, emerging evidence suggests heterogeneity among spliceosomes within cells. We hypothesize that this heterogeneity is regulated during critical cellular processes like differentiation and may be disrupted in pathological conditions such as cancer.
Methods: We analyzed publicly available human RiboMethSeq data to map 2'-O-methylation sites across snRNAs, and TGIRT-Seq and small RNA-Seq data to quantify snRNA variants and their expression levels. This approach allowed us to compare 2'-O-methylation patterns and snRNA variant abundance across cell types, tissues, and tumors. We employed RT-qPCR to validate key findings.
Results: U5 snRNA exhibit dynamic regulation during cell differentiation, with specific variants changing in abundance between pluripotent and differentiated states. Notably, these variants are also upregulated in various cancer cell lines. Additionally, we identified specific 2'-O-methylation sites that exhibit consistent changes across pluripotent cells, tissues, and cancer cells.
Discussion: Our study lays the groundwork for future research into the functional implications of spliceosome heterogeneity and its potential regulatory role in gene expression during cellular differentiation and cancer.
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Deciphering the Potential Role of the IVS-Loop in the Voltage-Sensing Mechanism of Prestin in Mammalian Hearing. Tiaren Ruiz Rojas[1], Nicolas Fuentes-Ugarte[2], Camila Pizarro[1,3], Víctor Castro-Fernandez[2] and Raúl Araya-Secchi[1,4] (raul@uss.cl). [1]Computational Biophysics group, Facultad de Ingeniería, Tecnología y Diseño, Universidad San Sebastián, Santiago, Chile. [2]Escuela de Bioquímica, Facultad de Medicina y Ciencia, Universidad San Sebastián, Santiago, Chile. [3]Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile. [4]Centro Basal Ciencia & Vida, Universidad San Sebastián, Santiago, Chile.

Introduction: The exceptional sensitivity and frequency selectivity of the mammalian hearing organ is primarily the result of cochlear amplification, a process powered by the electromotility (EM) of cochlear outer hair cells (OHCs). EM is driven by prestin, a membrane protein from the SLC26 family that functions as a voltage-dependent area motor. While prestin is critical for mammalian hearing, its non-mammalian counterparts act as anion transporters and lack specialized area-motor functions. This research explores the evolutionary adaptations that transformed prestin from an ancestral transporter to an area-motor protein, with a particular focus on the intervening sequence (IVS) loop within the STAS domain-a region that has been largely overlooked but may play a crucial role in fine-tuning the sensing or response of prestin to changes in voltage.
Materials and Methods: Through ancestral sequence reconstruction, structural modeling, and molecular dynamics simulations, we explored the structure, dynamics, and interactions of the IVS loop in mammalian and non-mammalian prestin ancestors and representatives.
Results: Our findings suggest that the IVS loop in placental mammals directly interacts with residues near the intracellular cavity of the adjacent monomer, potentially modulating the response of prestin to voltage changes. These interactions could represent a novel mechanism of voltage sensing, distinct from those observed in other proteins.
Discussion: Structural and functional modifications in the IVS loop appear to have been pivotal in reducing ion transport capability while enhancing voltage sensitivity, thereby fine-tuning the motor function essential for cochlear amplification in mammals.
Acknowledgments: This work is supported by FONDECYT N°1231164 (RAS, TRR), Proyecto CCTE Ciencia y Vida Basal FB210008 (RAS), FONDECYT 1221667 (VCF) and Beca doctorado nacional ANID 21221449 (NFU).

Exploring the diverse world of small RNAs in fungi. Nathan R. Johnson[1,2] (nathan.johnson@umayor.cl), Lorena Melet[2,3],Fabian Gonzalez[2],Barbara Bernal[2].[1]Millennium Science Initiative, Millennium Institute for Integrative Biology (iBio), Santiago, Chile. [2]Centro de Genómica y Bioinformática, Facultad de Ciencias, Ingeniería y Tecnología, Universidad Mayor, Santiago, Chile. [3]Programa Doctorado en Genomica Integrativa, Facultad de Ciencias, Ingeniería y Tecnología, Universidad Mayor, Santiago, Chile.

Small RNAs (sRNAs) are the functional element of this RNA interference and they have been shown to be important in wide-ranging processes, including gene-regulation, defense, and genomic integrity. This process is ancient and common throughout eukaryotic organisms, where most of the research focus has been on plants and animals. Fungi also produce sRNAs, though comparatively little is known about how they function, where they are found, and their fundamental genomic characteristics. This work explores these elements in fungi, making use of sRNA-seq data from over 200 projects in 86 species to build a comprehensive understanding of sRNA- expressing loci. To develop this process, we built a custom pipeline for producing annotations in difficult organisms, addressing serious challenges associated with high-noise samples as are found in fungi. By utilizing careful thresholding, we can discretely define loci in these samples, allowing us to discern what are small RNAs or just noise. We find that fungi produce sRNAs in a unique spectrum of sizes, dependent on the organism. Similarly, we find that the Dicer proteins responsible for this process are unique in fungi, containing domains distinct from other homologs. We find great variability in the size and shape of loci, with comparatively small counts even when considering the small genome sizes compared to other eukaryotes. Considering the evolutionary retention of this process, this points important roles for these few loci. Analyzing miRNAs, we find few that meet the critical standards found in other eukaryotes, though those point to some level of conservation and hinting at an alternative locus type in fungi. While select roles are known in fungi, we are building a comprehensive understanding of what this ancient and important process looks like in distinct organisms. **Funding**: This work was supported by the National Agency for Research and Development of Chile (ANID) FONDECYT program (number 11220727); and the ANID - Millennium Science Initiative Program (ICN17_022).

ORAL SESSIONS ABSTRACTS

Oral session 1

1. Natural polyphenols acting as collagen cross-linkers: an opportunity to create collagen-based biomaterials. Carolina Añazco (carolina.anazco@uss.cl)[1], Jessica Molina[1], Miltha Hidalgo[2], Omar Porras[2], Felipe Oyarzún-Ampuero[3], Daniel Marques de Sá e Silva[4], Christina Virgiliou[4], Petros Pousinis[4], Helen Gika[4] and Giorgios Theodoridis[4]. [1]Nutritional Biochemistry Laboratory, School of Nutrition and Dietetics, Faculty of Health Care Sciences, Universidad San Sebastián, Valdivia, Chile. [2]Instituto de Nutrición y Tecnología de los Alimentos, Universidad de Chile, Santiago, Chile. [3]Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile. [4]Biomic_AUTh, Center for Interdisciplinary Research and Innovation (CIRI-AUTh), Balkan Center, Thessaloniki, Greece.

Introduction: Grape pomaces are rich in minerals, dietary fiber, bioactive compounds, and polyphenols, which can aid in the oxidative deamination of lysine residues in proteins like collagen. We tested the lysyl oxidase-like activity of grape pomace extracts (GPE) from the Pais grape strain on different substrates and identified the polyphenolic composition of skins and seeds. Then, we investigated the ultrastructural changes and cross-linking levels in the secreted dermal extracellular matrix (ECM) treated with non-cytotoxic doses of GPE.
Materials and methods: We used a fluorometric assay to test the enzymatic activity of GPE. β-APN was used as a selective inhibitor of LOXL2 activity. We analyzed polyphenol-enriched fractions from GPE collected in different years using UHPLC-MS/MS. Using SEM and immunodetection assays, we examined the cross-linking levels of decellularized ECM derived from human dermal ECM.
Results: GPE contains polyphenols that could be used as a natural cross-linker for the development of collagen-based biomaterials. GPE demonstrate to exhibit a lysyl oxidase-like activity, remain unaffected by LOXL2 inhibitors and possess a high concentration of some catechol-type polyphenols. We found a specific range of concentrations of GPE that improve collagen cross-linking in the human dermal ECM.
Discussion: Our study aimed to create a reinforced dermal matrix for potential applications in natural collagen-based biomaterials by altering collagen cross-linking through amine oxidative mechanisms.
Acknowledgment: FONDECYT 1212026 and USS-FIN-24-PASI-03.

2. Top7 is a metamorphic protein. Camila Graziele Corrêa[1, 2] (camila.graziele@postgrado.uv.cl), Are Mjaavatten[3] †, Javiera Martínez Bilbao[1,4], Maximiliano F. Figueroa[5], Christian A.M. Wilson[1]. [1]Laboratory of Biochemistry and Mechanobiology of Individual Molecules, Department of Biochemistry and Molecular Biology, Faculty of Chemical Pharmaceutical Sciences, Universidad de Chile, Santiago,

Chile. [2]Faculty of Science, Universidad de Valparaíso, Valparaíso, Chile. [3]University of South-Eastern Norway, Porsgrunn, Norway. [4]Advanced Center for Chronic Diseases (ACCDiS), Faculty of Chemical and Pharmaceutical Sciences, Universidad de Chile, Santiago, Chile. [5]Department of Biochemistry and Molecular Biology, Faculty of Biological Sciences, Universidad de Concepción.

Top7 is an artificial globular protein with 93 amino acids designed completely *ab initio*. It has been proposed that this protein can assume a metamorphic behavior, meaning it can adopt different conformations in the folded state. Furthermore, single-molecule studies elucidate variation in Top7 unfolding dynamics under various experimental conditions, revealing intermediates often obscured in bulk studies. However, studies on the behavior of this protein at different temperatures and stretching velocities the single-molecule level remain limited. Experiments with temperature variation are very relevant because they can elucidate the influence of this parameter on the unfolding/refolding processes, potentially showing conformational changes and determining the protein's energy profile. In this study, we subjected Top7 to mechanical forces using optical tweezers and monitored its unfolding and refolding at different temperatures (4°-7°, 7°-14°, 14°-20° and 20°-27°) and stretching velocities (10nm/s, 100nm/s, and 1000nm/s). Our findings reveal that Top7 unfolds under two distinct conformations at 4°-14° temperatures and fast stretch/relax speeds (1000 nm/s). Observamos que de fato o Top7 se comporta como uma proteína metamórfica em condições experimentais específicas. The equilibrium Gibbs free energy change (ΔG) values are similar to those obtained in Circular Dichroism experiments. An additional mechanical experiment was conducted by adding free Top7 in solution; to verify any potential protein-protein interactions, our results show a significant difference in the average unfolding force compared with Top7 alone, indicating that Top7 in solution may affect the conformation of the protein being unfolded and refolded. This study highlights the potential of exploring artificial protein unfolding dynamics to understand natural folding mechanisms and their broader biological implications.

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3. Screening of the selected plant extracts and their secondary metabolites from Solanaceae plant family against *a*-amylase & *a*-glucosidase using *in-vitro* and *in-silico* techniques. Muhammad Javid Iqbal[1,2] (mjavid.201705947@gcuf.edu.pk), Pía Loren[3], Luis A. Salazar[3]. [1]Faculty of Life Sciences, Government College University Faisalabad, Pakistan; [2]Doctoral Programme in Sciences, major in Applied Cellular and Molecular Biology, Universidad de La Frontera, Temuco, Chile; [3]Center of Molecular Biology and Pharmacogenetics, Department of Basic Sciences, Faculty of Medicine, Universidad de La Frontera, Temuco, Chile.

Introduction: Diabetes, particularly type 2 diabetes mellitus (T2DM), is a major health challenge and a leading cause of death worldwide. Current pharmaceuticals have limited success and often cause undesirable side effects. Herbal medicines offer a cost-effective alternative with fewer side effects. This study aims to evaluate the antidiabetic potential of selected plants from the Solanaceae family, known for its pharmacological diversity and historical use in folk medicine.
Methodology: Ten under-explored plants from the Solanaceae family were screened for their inhibitory effects on alpha-amylase and alpha-glucosidase, enzymes critical in managing T2DM. The extracts were tested in vitro to determine their dose-dependent inhibitory effects. IC50 values were calculated for the most effective extracts. Additionally, molecular docking studies were conducted to explore the binding interactions of over 100 phytochemicals with the active sites of the enzymes.
Results: The study found that all plant extracts, except *Solanum tuberosum, Solanum melongena,* and *Solanum lycopersicum*, inhibited alpha-amylase and alpha-glucosidase in a dose-dependent manner. *Capsicum annuum* (IC50 2.11 µg/ml) showed the highest inhibition of alphaglucosidase, while *Withania coagulans* (IC50 16.22 µg/ml) was most effective against alphaamylase. *Hyoscyamus niger* (seeds) was potent against both enzymes, with IC50 values of 35.85 µg/ml (alpha-glucosidase) and 44.56 µg/ml (alpha-amylase). Molecular docking identified Daturalactone 4, Grossamide, Pongamoside D, and Daucosterol as the top interacting phytochemicals, with Grossamide meeting all drug-like parameters according to Ro5.
Discussion: The study highlights the significant antidiabetic potential of certain Solanaceae family plants, particularly Hyoscyamus niger. These findings suggest that these plant extracts could be developed as novel, effective treatments for T2DM with fewer side effects compared to current pharmaceuticals. The identified phytochemicals, especially Grossamide, show promise as potential drug candidates, warranting further investigation.
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4. Optogenetic allosteric control of Casein Kinase 1a in *Neurospora crassa.* Roberto Sanhueza-Aballe (rsanhuezaaballe@uc.cl), José Ignacio Costa, Luis F. Larrondo. Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, iBio.

Introduction: The circadian clock of the filamentous fungus *Neurospora crassa* relies on a transcription-translation feedback loop (TTFL) where the negative element FRQ represses its own transcription. FRQ recruits Casein Kinase 1a (CK1a), which phosphorylates FRQ in a slow, time-dependent manner, allowing it to transition from a strong repressor to a weak one, time at which a new cycle of the TTFL can start. CK1a, while interacting with FRQ, phosphorylates and inhibit the positive elements (Transcription Factors) that control *frq* expression. The role of CK1a in the clockworks, is highly conserved from fungi and humans.

However, the exact role of phosphorylation at different times of the day is not fully understood. This work aims to provide insight into CK1a's circadian function by dynamically modulating its enzymatic activity using LightR, an optogenetic allosteric switch which has been successfully implemented in eukaryotic kinases (Shaaya et al. 2020 PMID: 32965214).
Materials and Methods: Following molecular dynamic simulation, we defined different sites where to insert LightR in Neurospora's CK1a sequence. The circadian phenotype under various light regimes was assessed using a bioluminescent circadian reporter. In addition, CK1a's essential functions were evaluated by comparing the growth rates of LightR strains in constant light and darkness.
Results: Strains incorporating the LightR switch at CK1a positions I32 and K163 exhibit significantly faster growth rates in constant light compared to constant darkness. The expression of *frq* in these strains is rhythmic under light-dark cycles but not in constant darkness.
Discussion: These results show that *N. crassa* mutants incorporating the LightR switch in CK1a are viable, confirming that the enzyme is active and that, moreover, its enzymatic activity is being actively regulated by light. Our data also suggest that LightR modulation of CK1 activity can alter circadian parameters. We are currently conducting experiments to further determine the levels and phosphorylation profile of FRQ in both light and darkness, such that we can better correlate overt and molecular phenotypes.
Acknowledgment: FONDECYT 1211715, ICM-iBIO.

5. Artificial cell-derived vesicles: innovative NK-extrusome for lung cancer treatment.
Javiera Carrasco-Rojas[1] (jacarrascor@udd.cl), Orlando Ramírez[1], Gabriela Zavala[1], Rafael Contreras[1], Felipe Sandoval[1], Belén Olivares[2], Miriam Larsen[3] Marit Inngjerdingen[3], José Antonio Jara-Sandoval[4,5], Christina Schuh[1]. [1]Centro de Medicina Regenerativa, Facultad de Medicina, Clínica Alemana-UDD, Santiago, Chile. [2]Centro de Química Médica, Facultad de Medicina, Clínica Alemana-UDD, Santiago, Chile. [3]Innate Lymphocytes and Cancer Laboratory, Department of Pharmacology, Institute of Clinical Medicine, University of Oslo. [4]Institute for Research in Dental Sciences, Faculty of Dentistry, Universidad de Chile, Santiago, Chile. [5]Department of toxicological and pharmacological Chemistry, Faculty of Chemical and Pharmaceutical Sciences, Universidad de Chile, Santiago, Chile.

Background: Lung cancer (LC) has the highest mortality rate. While targeted therapies are recommended, many patients use classical treatments, which have limitations. Extrusomes (EXT), artificial cell-derived vesicles, have emerged as a promising tool for overcoming these barriers. This study proposes a docetaxel-encapsulated EXT formulation generated from natural killer (NK) human-cells (EXT-NK-DTX) and evaluates their cytotoxic and internalization effects on LC cells.
Methods: EXT were generated by cell extrusion. Morphology was analyzed using TEM. Stability was determined via zeta potential. Composition was determine via proteomic analysis. The cytotoxic effect was assessed using MTT and caspase 3/7 activation assays.

Cellular uptake mechanisms were explored with pharmacological inhibition (membrane fusion, macropinocytosis, and clathrin/caveolae-mediated endocytosis).
Results: All vesicles displayed a stable cup-shaped and mean size <200 nm morphology. Proteomic analysis revealed differentially present proteins. A significant cytotoxic and apoptotic effect was observed with EXT-DTX treatments in LC cells. Internalization studies identified involvement of the 4 analyzed mechanisms.
Conclusion: All vesicles were fully characterized with stable morphology and differential composition. EXT-NK-DTX showed significant cytotoxic effects only in A549 cells. Four internalization mechanisms were identified in EXT-uptake.
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6. Detection of SARS-CoV-2 Neutralizing Antibodies in rheumatological patients in Atacama region, Chile. Maria Jose Gallardo-Nelson[1] (mariajose.gallardo@uda.cl), Marcos Cruces[2], Yolanda Gomez[3]. [1]Departamento de Medicina, Facultad de Medicina, Universidad de Atacama, Copiapó, Chile. [2]Policlínico Reumatología e Inmunología, Hospital Regional de Copiapó, Copiapó, Chile. [3]Departamento de Estadística, Facultad de Ciencias, Universidad del Bío-Bío, Concepción 4081112, Chile.

Introduction: Vaccination has been a fundamental part of managing the pandemic, however, data on the level of neutralizing antibodies (Nabs) in patients with rheumatological diseases is limited. The purpose of this study was to evaluate the immune response in adult patients with immunemediated rheumatic diseases (IMRDs) in Atacama.
Methods: We designed a longitudinal observational study conducted at the rheumatology department of Hospital de Copiapó. Nabs titers against the Wuhan and Omicron variant were analyzed between 1-20 weeks after administration of the fourth dose of the SARS-CoV-2 vaccine to 341 participants (218 IMRD patients and 123 healthy controls).
Results: The NAbs titer in patients and healthy controls presented high titers for both variants. A comparison between the Wuhan vs Omicron variants, we observed that there were significant differences ($p<0.05$) in the ID50 level, both for healthy controls and for patients. The humoral response of IMRD patients is significantly lower compared to healthy controls for the omicron variant ($p = 0.0015$). Furthermore, the response of patients with IMRD decreases significantly when the time interval between vaccination and sampling is greater than 35 days, in both variants. Treatments such as MTX, LEF, HCQ and biologics such as rituximab and abatacept demonstrated a significant decrease in humoral response in patients with IMRD.
Conclusion: Studying vaccination responses in immunosuppressed populations is crucial as their reactions differ from the general population. In IMRD patients, the humoral response to SARS-CoV-2 vaccines varied based on the dose, vaccine type, response times, and treatments used.
Acknowledgment: FONIS SA21I0078.

7. LEAP-2/Ghrelin Signaling in Palmitic Acid-Induced Cardiomyocyte Hypertrophy and Metabolism. Sebastian Aedo-Cares[1,2,3] (seba.aedo.cares@gmail.com), Sebastián Leiva-Navarrete[1,2], Rodrigo Troncoso[2,3,*], Valentina Parra[1,2,4,*]. [1]Laboratory for Cell Differentiation and Metabolism, Department of Biochemistry and Molecular Biology, Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile. [2]Advanced Center of Chronic Diseases (ACCDiS), Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile. [3]Laboratorio de Investigación en Nutrición y Actividad Física, Instituto de Nutrición y Tecnología de los Alimentos, Universidad de Chile. [4]Systems Biology Center for the Study of Extremophile Communities from Mining Tailings (SYSTEMIX), O'Higgins University, Rancagua, Chile.

Introduction: Palmitic acid (PA), a saturated fatty acid prevalent in obesogenic Western diets, induces cardiomyocyte hypertrophy and hepatocyte steatosis. Patients with fatty liver disease are more prone to cardiovascular diseases. LEAP-2, a hepatokine with elevated plasma levels in obesity, has recently been identified as a competitive antagonist and inverse agonist of ghrelin and its receptor GHSR. Ghrelin is known for its cardioprotective effects in various cardiovascular diseases and hypertrophic stimuli. Our previous RNA-seq studies on high-fat diet animal livers indicated increased LEAP-2 levels. However, the role of LEAP-2 signaling in cardiomyocyte metabolism and PA-induced hypertrophy remains unclear. This study aimed to determine the effects of LEAP-2 signaling and LEAP-2/ghrelin interaction on PA-induced cardiomyocyte hypertrophy.
Materials and Methods: Neonatal rat ventricular cardiomyocytes (NRVCMs) were stimulated with LEAP-2; and either BSA-Control or BSA-conjugated PA, with or without LEAP-2 and ghrelin. We evaluated cellular area, hypertrophic and metabolic markers, and mitochondrial morphology using immunofluorescence, live cell microscopy, and qRT-PCR.
Results: LEAP-2 increased cardiomyocyte area and altered mRNA levels indicative of dysregulated lipid uptake (CD36) and mitochondrial incorporation (CPT1B). Ghrelin prevented PA-induced hypertrophy, reducing both cellular area and BNP mRNA levels. LEAP-2 did not affect PA-induced increases in cardiomyocyte area but inhibited the protective effects of ghrelin and appeared to regulate PA-induced hypertrophic markers and mitochondrial morphology changes.
Discussion: These findings suggest that an imbalance in the LEAP-2/ghrelin ratio in obesity is associated with altered cardiomyocyte lipid metabolism, exacerbating PA-induced hypertrophic responses. Our study, preliminarily underscores the importance of LEAP-2 and ghrelin signaling in cardiomyocyte hypertrophy and metabolism, highlighting potential therapeutic targets for obesity-related cardiac hypertrophy.
Funding: This project is funded by ANID FONDECYT 1230195 (VP), Anillo SYSTEMIX ACT210004 (VP), FONDAP 15130011 (RT, VP), and ANID PhD scholarship 21220767 (SA-C); Universidad de Chile grants Enlace FONDECYT ENL01/23 (RT) and Apoyo a la

Infraestructura para la Investigación INFRA037/2023 (VP); and National Institutes of Health (NIH) grant 2R01HL142302-05A1 (RT).

8. Histatin-1, a salivary peptide with regenerative capacity. Patricio Silva[1], Pedro Torres[1], Carlos Mateluna[1], Héctor Tapia[1], Luis Córdova[2], Floris Bikker[3], Gerald Zapata[4], Christian Wilson[5], Vicente A. Torres[1,5,6,7]. [1]Institute for Research in Dental Sciences, Faculty of Dentistry, Universidad de Chile, Santiago, Chile; [2]Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Universidad de Chile, Santiago, Chile; [3]Department of Oral Biochemistry, Academic Centre for Dentistry Amsterdam, VU University & University of Amsterdam, Netherlands; [4]Molecular Graphics Suite, Department of Inorganic and Analytical Chemistry, Faculty of Chemical and Pharmaceutical Sciences, Universidad de Chile, Santiago, Chile; [5]Department of Biochemistry and Molecular Biology, Faculty of Chemical and Pharmaceutical Sciences, Universidad de Chile, Santiago, Chile; [6]Advanced Center for Chronic Diseases (ACCDiS); [7]Millennium Institute on Immunology and Immunotherapy (MIII).

Histatins are salivary peptides that play important roles as antimicrobial peptides and contribute to maintaining tooth enamel homeostasis. From within this group of peptides, Histatin-1 stands out, as it has been described as a factor that promotes wound healing in different tissues, such as oral mucosa, skin, and bone tissue. The variety of tissue effects that Histatin-1 exerts is mainly due to its migration-promoting activity in many cell types, including keratinocytes, fibroblasts, pre-osteoblasts, and endothelial cells. Particularly, in the latter model, our group demonstrated that Histatin-1 is a novel pro-angiogenic factor that increases the adhesive and migratory capacity of endothelial cells and induces the formation of vascular tubes and angiogenesis *in vivo*. Following these landmarking studies, several reports have emerged indicating that the reparative capacity of Histatin-1 *in vivo* is largely due to its pro-angiogenic property. However, despite the knowledge about the mode of action of Histatin-1, the mechanism of action by which this molecule induces angiogenesis remained largely unknown. Recent studies from our group identified the receptor for Histatin-1 in endothelial cells, as the Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2). The direct binding of Histatin-1 and VEGFR-2 is responsible for the induction of endothelial cell migration and the activation of downstream signaling pathways. However, several questions remain unanswered regarding the extent of the effects that this molecule has, a scenario that is exemplified by recent findings indicating that Histatin-1 is a novel osteogenic factor, contributing to bone tissue repair. Many of these questions are currently being addressed by different research groups, including ours, which will favor the development of therapeutic strategies based on the usage of this molecule in regenerative medicine.
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Oral session 2

1. Homeostats–the hidden rulers of homeostasis and signaling in plants. Ingo Dreyer (idreyer@utalca.cl), Center of Bioinformatics, Simulation and Modeling (CBSM), University of Talca, Chile.

Introduction: Ion homeostasis is a crucial process in plants that is closely linked to the efficiency of nutrient uptake, stress tolerance and overall plant growth and development. Recent modeling approaches have shown that it is not individual transporters but rather transporter networks (homeostats) that control membrane transport and associated homeostatic processes in plant cells closing a severe gap in our understanding of the fundamental processes of ion homeostasis.
Materials and Methods: To analyze the dynamic properties of transporter networks, an unbiased, systemic approach combined thermodynamics with biophysics and translated biological phenomena into the language of mathematics. This enabled the derivation of analytical solutions or computational simulations of specific situations.
Results: The analyses and simulations allowed deep unprecedented insight into the dynamics and coordination of ion transport by transporter networks. Homeostats do not only control ion and hormone (auxin) homeostasis, but are also a hidden prerequisite for electrical, hydraulic, pH and Ca^{2+}-signaling in plants.
Discussion: Transporter networks that act together exhibit dynamic properties as a system that go beyond of those of the isolated transporters.
Acknowledgment: FONDECYT 1220504, Anillo ATE220043.

2. Functional Expression of Pectin Methylesterase (PME) from Chilean Papaya in the Heterologous System *Pichia pastoris*. Paloma Fuenzalida[1] (paloma.fuenzalida@ug.uchile.cl), María Paz Covarrubias[1], Dayan Sanhueza[2], Susana Saez[2], Tamara Méndez[3], Raúl Herrera[3], Michael Handford[1]. [1]Centro de Biología Molecular Vegetal, Departamento de Biología, Facultad de Ciencias, Universidad de Chile. [2]Centro de Biotecnología Vegetal, Universidad Andrés Bello. [3]Laboratorio de Fisiología Vegetal y Genética Molecular, Instituto de Ciencias Biológicas, Universidad de Talca.

The Chilean agroindustry plays a crucial role in fruit processing, generating substantial amounts of plant residues, primarily consisting of plant cell wall (CW) fibers. To manage these residues, robust CW-degrading enzymes could be utilized to dismantle these structures. *Vasconcellea pubescens*, or Chilean mountain papaya, is known for its abundant proteases and highly acidic pH in the flesh, which may endow its CW-degrading enzymes with unique properties suitable for biotechnological applications. Pectin methylesterase (PME) types I and II are key enzymes that remodel the plant CW by removing methyl groups from homogalacturonan, leaving it susceptible to degradation by other enzymes to produce oligogalacturonides, carbohydrates with beneficial biological properties. In this study, PME1

and PME2 were identified from the transcriptome of *V. pubescens*. Three-dimensional models of the predicted proteins revealed the presence of the characteristic domains for both PME types. The corresponding genes were amplified and cloned into the pPICZαA vector for expression in *Pichia pastoris*. The presence of these genes in the transformed *P. pastoris* colonies was verified by PCR. Methanol induction successfully drove the expression of VpPME1 and VpPME2, as verified by dot blot and western blot analyses, which revealed the presence of recombinant proteins in the culture medium. Subsequent analysis of the culture medium demonstrated significant PME activity, indicating that the expressed enzymes are functionally active. These findings highlight the potential of VpPME1 and VpPME2 for their use in enzyme cocktails aimed at treating agro-industrial waste and generating beneficial health products.
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3. *Arabidopsis thaliana* transcription factors bZIPs C/S1 network and their role regulating the response to nitrate starvation. Carolina Galleguillos (carogalleguillos@gmail.com), Nicolás Nahuel and Lorena Norambuena. Center of Molecular Biology in Plants, Department of Biology, Faculty of Science, Universidad de Chile, Santiago, Chile.

Basic leucine-zipper (bZIP) transcription factors are master regulators of responses to different stimuli in eukaryotes. In plants, bZIPs have been studied mainly for their role in seed maturation, flowering, and response to biotic stress, light, and abiotic stress. In *Arabidopsis thaliana*, 78 bZIPs have been identified and classified into 13 groups. In our laboratory, we have observed that the loss of function of genes of the C/S1 network of bZIPs exhibits an increased endocytic trafficking, which may impact on phosphate and nitrogen uptake. Therefore, we hypothesize that these bZIPs would have a role in the regulation of genes related to the response to nutrient deficiency. Thus, we performed a phenotypic characterization of the loss of function mutants under nitrate starvation stress. The loss-of-function mutants of genes encoding for the transcription factors bZIP1, bZIP10, bZIP25 and bZIP63 did not modify the root structure in response to nitrogen starvation as the *Arabidopsis thaliana* wild ecotype. The key regulator that plays an important role in controlling nitrate uptake *NRT2.1* is upregulated in response to nitrogen starvation. In *bZIP1*, *bZIP25*, and *bZIP63* loss-of-function mutants, *NRT2.1* transcript levels were up-regulated under control conditions, suggesting that these transcription factors may be negatively involved in the nitrogen starvation response. Consistent with this, transcript levels of the *bZIP1*, *bZIP25*, and *bZIP63* genes are down-regulated in response to nitrogen starvation in the wild-type *Arabidopsis*. This regulation seems to be specific since we had not detect alteration on the response of the loss of function mutant of *bZIP1*, *bZIP25*, and *bZIP63* to phosphate deficit.

Overall, our results place *bZIP1*, *bZIP25*, and *bZIP63* as important players in the response to nitrogen deficit in *Arabidopsis*.
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4. Transcription Factors involved in root development under low phosphate and salt combined stress. Hernán Grenett-Salinas[1,3], Miguel Ángel Ibeas[1,2,3,4], Tomás Moyano[1,3], Nathan Jhonson[3,5], Romina Acha-Escobar[1,2,], Jorge Pérez[1,2], Thomas Muñoz-Duman[1], Elena Vidal[3,5,6], José Miguel Álvarez[1,3], José Manuel Estévez[1,2,3,4]. [1]Centro de Biotecnología Vegetal, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago, Chile. [2]ANID-Millennium Science Initiative Program – Millennium Nucleus for Development of Super Adaptable Plants (MN-SAP), Santiago, Chile. [3]ANID-Millennium Science Initiative Program – Millennium Institute for Integrative Biology (iBio), Santiago, Chile. [4]Fundación Instituto Leloir and IIBBA-CONICET, Buenos Aires, Argentina. [5]Centro de Genómica y Bioinformática, Facultad de Ciencias, Ingeniería y Tecnología, Universidad Mayor, Santiago, Chile. [6]Escuela de Biotecnología, Facultad de Ciencias, Ingeniería y Tecnología, Universidad Mayor, Santiago, Chile.

Introduction: Climate change has generated significant variations in soils, leading to nutrient deficiency and biotic stress that affect the plant's development. An example is the deficiency of phosphate (Pi), a macronutrient essential for plant life. On the other hand, salt stress is one of the main stresses that cause cellular damage. To date, the mechanisms of mitigation and signaling to low Pi and salt stresses are known, but signals have been studied in an independent manner. The main organ in plants that oversees nutrient capture and is also the primary barrier in the soil for stress is the root, composed of the primary, lateral, and root hairs. While the presence of these stresses occurs simultaneously during the life of plants, there is little information on the molecular pathways that regulate this multi-stress response. In this work, we have coupled a data mining strategy with phenotypic analysis to identify and characterize new transcription factors (TFs) that participate in the root system architecture (RSA) remodeling during this dual stress.
Materials and Methods: We mined the available information in transcriptomics and experimental datasets of Pi and salt stress. In addition, we searched for protein-protein interaction of TFs using Alfa Fold (AF). This information allowed us to obtain a list of TF candidates validated phenotypically in insertional mutants for each TF in the presence of the combined stress and analyze the RSA changes.
Results: We identified at least ten candidate TFs that participate in the RSA remodeling. Among them, bZIP2, ABF2, and HB6 shows protein-protein interactions. TF mutants submitted to the dual stress show different RSA trait changes compared to wild-type plants.
Discussion: Taken together, our findings reveal that *ABF2* and *HB6* are regulators of the phenotypic response of Arabidopsis root to low Pi and salt and provide new insights into the complex regulatory networks of plant acclimation to stress combination.

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5. Nitrate and CK interplay in modulating leaf growth in *Arabidopsis thaliana*. Liliana Lamig (lalamig@uc.cl), Rodrigo A. Gutiérrez. Millennium Institute for Integrative Biology (iBio), Millennium Institute Center for Genome Regulation (CRG), Institute of Ecology and Biodiversity (IEB), Facultad de Ciencias Biológicas-Pontificia Universidad Católica de Chile.

Introduction: Nitrate – the major source of N for plants in agricultural soils – is considered a signal molecule that regulates plant growth and development. Although nitrate is perceived primarily in the roots, it also affects above-ground organ growth, such as leaves. Nitrate enhances leaf growth mainly by promoting cell expansion and endoreplication – a variant of the mitotic cell cycle where ploidy levels (nuclear DNA content) increase without cell division. The phytohormone cytokinin (CK) acts as a long-distance signal mediating several nitrate-induced effects on shoot growth and development. Also, it modulates ploidy levels and cell size in the leaf epidermis, thereby impacting the final leaf size.
Materials and methods: We analyzed leaf size, ploidy, and epidermal cell size during early post-germinative growth in *Arabidopsis thaliana* in response to varying CK levels and nitrate regimes. These analyses were conducted using reverse genetics, flow cytometry, and confocal microscopy. In addition, we assessed the impact of nitrate availability on the CK response in the leaf epidermis using a CK reporter line and confocal microscopy.
Results: We found that altering CK levels affects nitrate-induced cell expansion and endoreplication. Furthermore, we observed that nitrate influences the CK dynamics in the leaf epidermis.
Discussion: We propose that nitrate modulates endoreplication through CK dynamics, thereby fine-tuning cell expansion and, ultimately, influencing leaf size.
Acknowledgments: ANID–Millennium Science Initiative Program-Millennium Institute for Integrative Biology (iBio) (ICN17_022), the Center for Genome Regulation (ICN2021_044), and ANID-FONDECYT 1220594.

6. N-glycosylations from development through postharvest in commercial strawberry (*Fragaria x ananassa*). Angela Méndez-Yáñez [1](angela.mendez@uautonoma.cl), Darwin Sáez [1,2], Francisca Rodríguez-Arriaza [1], Daniel Bustos [3,4], Gabriela Urra [3], Ricardo Castro [5], Marcelo Muñoz-Vera [5], Matías Moreno-Bertolone [1], Claudio Letelier-Naritelli [1], Yessica Burgos-Rojas [1], Luis Morales-Quintana[1]. [1]Multidisciplinary Agroindustry Research Laboratory, Instituto de Ciencias Biomédicas, Facultad de Ciencias de la Salud, Universidad Autónoma de Chile, Talca, Chile. [2]Programa de Doctorado en Ciencias Biomédicas, Instituto de Ciencias Biomédicas, Facultad de Ciencias de la Salud, Universidad Autónoma de Chile,

Talca, Chile. [3]Laboratorio de Bioinformática y Química Computacional, Departamento de Medicina Traslacional, Facultad de Medicina, Universidad Católica Del Maule, Talca, Chile. [4]Vicerrectoría de Investigación y Postgrado, Universidad Católica Del Maule, Talca, Chile. [5]Multidisciplinary Agroindustry Research Laboratory, Instituto de Ciencias Aplicadas, Facultad de Arquitectura y Construcción, Universidad Autónoma de Chile, Talca, Chile.

Introduction: More than 50% of the proteins of plant cell wall are N-glycosylated, where this post-translational modification is involved in folding and structural stability. Throughout development and fruit ripening, both the N-glycosylation sites (N-glycosites) and the N-glycans that compose N-glycosylation change depending on the tissue and stage. N-glycans are cleaved and released by exoglycosidases, which act as signal molecules promoting fruit ripening. Previous studies have shown that RNAi of the exoglycosidases α-mannosidase (α-man) and β-D-N-acetylhexosaminidase (β-hex) in tomatoes can extend fruit shelf life by up to 30 days. The aim of this study was to screen N-glycosylation from development through postharvest stages using commercial strawberries as a model (*Fragaria x ananassa*), with focus in α-man and β-hex exoglycosidases.
Materials and Methods: Relative expression of gene family of *α-man* and *β-hex* and total enzyme activity were realized. Temporary inhibition of N-glycosylation was performed from white to ripe developmental stages. Postharvest treatments with ClO_2 were applied to ripe fruits, which were then stored at 4ºC for 9 days. Data mining of the *Fragaria x ananassa* genome was conducted to identify all sequences of *α-man* and *β-hex* gene families to analyze *in silico*.
Results: Differential expression patterns were observed in both gene families, with total enzyme activity decreasing in *α-man*, conversely with *β-hex* across developmental and ripening stages. Inhibition of N-glycosylation demonstrated a loss of enzyme activity at 50% ripening stage in both enzymes, along with changes in cell wall rearragement. ClO_2 treatments evidenced changes in the activity of both exoglycosidases. *In silico* results revealed specific amino acid patterns flanking the sequon, showing a preference for R group charge; loops were identified as the primary localization of N-glycosites, and a variable number of potentially N-glycosites were observed, depending of the protein.
Discussion: N-glycosylations along with the role of both exoglycosidases from development through postharvest of commercial strawberries is discussed.
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7. Molecular and functional validation of edited apple plant prototypes to reduce enzymatic browning and increase carotenoids content. Samuel Parra[1] (samuel.parra@me.com), Claudia Cardenas[1], Christian Gonzalez-Calquin[1], Leticia Amaza[1] and Claudia Stange[1]. [1]Plant Molecular Biology Centre, Department of Biology, Faculty of Sciences, Universidad de Chile, Ñuñoa, Santiago, Chile.

Introduction: Chile is the fourth largest exporter of apples worldwide and first in the Southern Hemisphere, mainly Fuji and Royal Gala. However, 35% of apple orchards are obsolete requiring an urgent varietal renewal, hopefully in traits that will give value to consumers and the industry. Enzymatic browning (EB) caused by polyphenol oxidase (PPO) enzymes is one of the main discourages of apple consumption, reducing its shelf life as fresh fruit, fresh-cut products, and juices, affecting flavor, nutritional value, and generating enormous food waste. On the other hand, apples have low levels of provitamin A given by carotenoids, pigments with attractive color and antioxidant capacity. To take advantage, a joint venture between Universidad de Chile and its partners Biofrutales and Los Olmos nursery developed Fuji Raku Raku and Royal Gala apple plants edited with CRISPR/Cas9 for *CCD4* and *PPO* genes to increase carotenoids and decrease pulp EB, respectively, obtaining the first government permit to plant edited fruit trees in Chile.
Methods: In this work, plants were characterized using DNA sequencing to determine edited and not transgenic individuals. PPO activity was measured using enzymatic assays and carotenoid content was determined spectrophotometrically.
Results: Ninety-seven plants were functional and molecular analyzed, selecting thirty-five edited non-GMO plants with 5% to 55% gene editing. Phenotypic analysis of this trees showed reduced PPO activity and double of total carotenoid content in leaves.
Discussion: Gene edited crops are more accepted than genetically modified crops although screening to obtain non-GMO edited plants is a complex task The methodology applied in this work has been successfully in obtaining 6% of edited non-GMO apple trees with reduced EB and more carotenoid content in leaves which we expect to be reflected in the first fruits to be obtained next season.
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8. Modulation of volatile production in strawberries fruits by endophytic fungi. Patricio Ramos[1](pramos@utalca.cl), Francisca Rodríguez-Arriaza[1], Mariona Gil i Cortiella[2], Stephan Pollmann[3], Luis Morales-Quintana[4]. [1]Instituto de Ciencias Biológicas, Universidad de Talca. [2]Instituto de Ciencias Aplicadas, Universidad Autónoma de Chile. [3]Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid. [4]Instituto de Ciencias Biomédicas, Universidad Autónoma de Chile.

Introduction: Strawberries (*Fragaria* x *ananassa*) are valued worldwide for their exceptional aroma, flavor, color, and nutritional benefits. Pyruvate decarboxylase (PDC) is a key enzyme in fruit quality, and is essential in ethanol metabolism, leading to the formation of esters and

other aromatic compounds that contribute to the unique aroma of strawberries. Additionally, alcohol acyltransferases (AATs) play significant roles in acyl group transfer, enhancing the aroma diversity of strawberries. However, strawberries are highly susceptible to drought, which affects their quality including aroma. Plant root-associated fungi offer a novel solution to mitigate water deficiency stress in different plants including strawberry.
Materials and methods: This study examines the impact of Antarctic fungal inoculation on expression profiles of *FaPDC* and the *FaAAT* gene families, related to the volatile organic compounds (VOCs) production in fruit of strawberry plants under drought stress. VOCs profile and total AAT enzymatic activity were evaluated.
Results: Under drought stress, fruits from fungi-inoculated plants exhibited significant changes in gene expression, increasing the total volatile esters, particularly acetate esters. Additionally, total activity of AAT increased under drought in fruit of inoculated plants.
Discussion: These findings highlight the role of Antarctic fungi in modulating the metabolic pathway of VOCs by inducing the expression of *FaPDC* and *FaAAT* genes. The study underscores the potential of Antarctic microorganisms as valuable tools to maintain and restore the sensory attributes of strawberries under water deficiency enhancing the aromatic compounds biosynthesis.
Acknowledgment: FONDECYT 1240771, 1220782, 1211057. ANILLO ATE220014.

Oral session 3

1. Tissue-specific gene regulatory network models from large-scale datasets enable discovery of novel regulators of key biological processes in *Solanum lycopersicum*.
José D. Fernández[1,2] (jose.fernandezpe@mayor.cl), David Navarro[5], Javier Canales[2,3], José M. Álvarez[2,4], José Tomás Matus[5], Elena A. Vidal[1,2]. [1]Centro de Genómica y Bioinformática, Universidad Mayor, Chile. [2]ANID-Millennium Science Initiative Program-Millennium Institute for Integrative Biology (iBio), Chile. [3]Instituto de Bioquímica y Microbiología, Universidad Austral de Chile, Chile. [4]Centro de Biotecnología Vegetal, Universidad Andrés Bello, Chile. [5]Instituto de Biología Integrativa de Sistemas I2SysBio, España.

Introduction: Tomato (*Solanum lycopersicum*) is a widely grown crop, relevant to the human diet, and is considered a model organism for fruit development and response to plant pathogens. Despite its significance, there is still a lack of information about how gene expression is orchestrated by transcription factors (TFs) in this species, which is key for understanding the regulation of biological and molecular processes and for proposing strategies to improve tomato productivity and adaptation to the environment.
Materials and Methods: We developed five organ-specific reference Gene Regulatory Networks (rGRNs) for tomato by integrating updated gene structural and functional annotations, TF-target interactions obtained from random forest-based inferences from more than 10,000 RNA-seq libraries, and other information sources, including gene co-

expression networks, chromatin accessibility datasets and bioinformatic prediction of TF genomic binding sites.
Results: We were able to obtain rGRNs representing TF-target regulatory interactions for tomato roots, leaves, flowers, seeds and fruits. Our results indicate that while expression of genes is relatively consistent between different tissues, regulatory interactions are highly tissue-specific, with different central TF regulators coordinating main biological processes across tissues. As a proof of concept, we used our regulatory predictions to gain new insights on the fruit ripening regulatory cascade, a well-studied process at the physiological and molecular levels. The fruit rGRN, was able to recapitulate known and experimentally validated targets of the central ripening controllers TAGL1, CNR, and RIN, validating our approach. We used the regulatory information of the rGRN to infer new TFs controlling ripening-related genes, finding a key TF from the ARF family as potential central controller of ripening, in particular of genes involved in carotenoid metabolism, cell wall development, ethylene and ABA signaling.
Discussion: Our tissue-specific rGRNs provide a valuable framework for identifying novel central regulators of tomato development and response to the environment.
Acknowledgments: ANID-FONDECYT 1211130, 1230833, 1210389, ANID-Millennium Science Initiative Program ICN17_022, ANID-Anillo ACT210007, FOVI230159 and Beca Doctoral ANID 21230478.

2. Mimicking the toothbrushing bacterial detachment through multiscale SIRAH simulations. Ivana Orellana[1] (ivana.orellana01@mayor.cl), Sergio Pantano[2] , Andrés Rivas-Pardo[1]. [1] Mechano Biology Group, Microbe Genomics lab, Center for Genomics and Bioinformatics, Universidad Mayor, Santiago, Chile. [2] Biomolecular Simulation Group, Instituto Pasteur of Montevideo, Montevideo 11400, Uruguay.

Introduction: Dental cavities are one of the most widely distributed human diseases. According to WHO, more than 90% of the world population is currently infected with *Streptococcus mutans*, an etiological agent responsible for human cavities. To successfully accomplish the colonization of the dental tissue, oral bacteria use large protein filaments to secure their attachment. Pili, as these molecular complexes are known, mediate the interaction of the bacteria with the dental surface, withstanding large mechanical forces imposed by the host, including saliva flow and tooth brushing. Computational tools, including molecular dynamics simulations, allow us to simulate and predict the mechanical behavior of pili proteins under force. However, due to the large number of atoms included in the molecular complexes, molecular dynamics has been relegated to simulating only short times and forces above the physiological range that *S. mutans* experienced *in vivo*.
Material and Methods: We conduct molecular dynamics using the GROMACS, including the multiscale SIRAH force field, which allows coarse-grained and supra-coarse-grained simulations.

Results: Ag I/II, the main elastic component of the *S. mutans* pili protein, is made of 486 residues which are organized in three independent immunoglobulin-like domains. Each domain is structurally stabilized by the presence of an isopeptide bond, critical for preventing the mechanical unfolding.Keeping the protein and solvent under coarse-grained and supra-coarse-grained representation, we were able to decrease the number of atoms of the entire molecular system to less than a tenth, compared to full atomistic models. This decrease in the number of atoms saves computational resources and allows us to implement steered molecular dynamics that are close to the physiological forces that *S. mutans* experienced during surface attachment.
Discussion: Our results demonstrate that it is possible to simulate physiological forces for the AgI/II bacterial adhesion using multiscale SIRAH molecular dynamics.
Acknowledgment: FONDECYT1221064 (ARP), ANID21221055 and UNUBIOLAC(IO).

3. Identification and characterization of novel lncRNAs in *R. norvegicus* cardiac fibroblasts. Allan Peñaloza-Otárola[1] (allan.penaloza@ug.uchile.cl), Sebastián Urquiza-Zurich[1], Francisco Sigcho-Garrido[1], Danica Jiménez-Gallegos[1], Elsa Rocio Bascuñan[1], Sebastián Leiva-Navarrete[1], David Silva-Ancahuail[1], Sergio Lavandero[1 2], Vinicius Maracaja-Coutinho[1 2]. [1]Advanced Center of Chronic Diseases (ACCDiS), Faculty of Chemical and Pharmaceutical Sciences & Faculty of Medicine, University of Chile, Chile. [2]Cardiology Division, Unversity of Texas Southwestern Medical Center, Dallas, USA.

Introduction: Cardiac fibrosis is critical pathological remodeling process in heart failure, which is characterized by excessive accumulation of extracellular matrix and proliferation of myofibroblasts. Long non-coding RNAs have recently been described as key regulators in the development of cardiac diseases. However, their roles in cardiac fibrosis are still not clear. Hereby, we focus on the identification and characterization of lncRNAs that may have key roles in regulating *Rattus norvegicus* cardiac fibrogenesis.
Methods: We downloaded R. norvegicus RNA-Seq data from five public datasets (PRJNA1090015, PRJNA1080202, PRJNA833249, PRJNA727096, and PRJNA554512) and applied a transcriptomics pipeline for the detection and annotation of novel transcripts. The pipeline includes quality control via FastQC, trimming the reads via Fastp, aligning with Hisat2, reconstructing transcripts via StringTie, and examining the potential coding capacity via RNAmining. The transcripts were further classified based on their genomic locations and compared with data from the Ensembl database using GffCompare.
Results: Of these reconstructed transcripts, 11,047 were of particular interest and thus categorized into 7,741 exact matches ('='), 1,599 intergenic ('u'), 628 intronic ('i'), and 1,079 exonic overlaps in the opposite strand ('x'). RNAmining analysis identified a huge number of putative novel noncoding lncRNAs. Noticeably, the lengths of transcripts differed by type; the median length for multi-exonic transcripts was 1,227 nucleotides, while for mono-exonic transcripts, it was 498 nucleotides.

Conclusions: Our results identified a significant proportion of putatively novel lncRNAs in neonatal *R. norvegicus* cardiac fibroblasts that have drastically expanded current knowledge regarding the landscape of noncoding RNA, uncovering large gaps in current genomic annotations. This work outlines the need for improvement in genomic annotation in *R. norvegicus* and provides the groundwork for further functional research.
Funding: FONDECYT 1211731 (VMC), 1240443 (SL), FONDAP 1523A0008 (VMC, SL)., AmSud STIC 2020008 (VCM), Anillo (ACT210004 and ATE220016 (VMC))

4. Identification of gene regulatory networks and key transcription factors controlling *Vitis vinifera* gene responses to drought. Gabriela Vásquez[1] (Gabriela.vasquez505@gmail.com), Tomás C. Moyano [1,3], José Miguel Álvarez [1,3], Ariel Orellana[1,2]. [1]Centro de Biotecnología Vegetal, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago, Chile. [2]Instituto Milenio Centro de Regulación del Genoma (CRG), Santiago, Chile. [3]Instituto Milenio de Biología Integrativa (iBio), Santiago, Chile.

Climate change is expected to intensify drought events in the future, posing significant challenges for agriculture. Grapevine (*Vitis vinifera*) stands as one of the most economically crucial crops globally, with nearly all wine-producing regions situated in temperate zones. To combat the adverse effects of drought, understanding and enhancing grapevine drought tolerance is imperative. The plant's response to drought involves changes in gene expression regulated by transcription factors (TFs) that can activate or repress genes in complex ways. Over the past 15 years, vast amounts of publicly available transcriptomic data have accumulated, providing an opportunity to explore the genetic basis of stress resistance.
This study utilized publicly available transcriptome datasets of *Vitis vinifera* to analyze differential expression between drought and control conditions. Gene regulatory networks were constructed by combining genome-wide motif-based analysis of promoter regions (FIMO) with the GENIE3 machine-learning algorithm to infer regulatory networks from transcriptomic data. This approach enabled the identification of key TFs involved in drought response by ranking them based on their regulatory importance.Despite the variability in transcriptomic data, a consistent core of drought-responsive genes was identified. Key TFs were ranked according to their magnitude of response, consistency across datasets, and regulatory influence. Known drought-responsive TFs from other plants, such as homeobox-leucine zipper protein 12 (HB-12), were found, validating our strategy. Additionally, novel TFs were identified as potential candidates for further validation. These TFs exhibited significant regulatory control over numerous drought-responsive genes, highlighting their critical roles in the adaptive response of *Vitis vinifera* to drought stress.
Identifying key TFs in *Vitis vinifera* highlights potential targets for enhancing drought tolerance through biotechnological interventions. Future work will focus on experimentally validating these TFs to gain a better understanding of drought tolerance mechanisms.

5. Matrin-3 promotes cap-independent translation initiation of the full-length HIV-1 mRNA. Jimmy Martínez-Torres[1] (Jamartinez9@uc.cl), Marcelo López-Lastra[1]. [1]Laboratorio de Virología Molecular, Instituto Milenio de Inmunología e Inmunoterapia, Departamento de Enfermedades Infecciosas e Inmunología Pediátrica, Escuela de Medicina, Pontificia Universidad Católica de Chile.

Introduction: The human immunodeficiency virus type 1 (HIV-1) is a member of the *Retroviridae* and the etiologic agent of acquired immunodeficiency syndrome (AIDS). During infection, the viral genetic material is permanently integrated into the host genome and is expressed by the host's transcription, RNA processing, and translation machinery. A proportion of the pre-mRNA subverts RNA processing, giving rise to the partially spliced and full-length HIV-1 RNAs, both recognized and transported to the cytoplasm by Rev, a viral nuclear-cytoplasmic shuttling RNA binding protein (RBP). The capped and polyadenylated full-length HIV-1 RNA (vRNA), harbors an IRES in its 5'UTR, the HIV-1 IRES. Thus, translation initiation of the HIV-1 vRNA can follow a cap- or internal ribosome entry site (IRES)-dependent mechanism. The HIV-1 IRES function is regulated by RBPs, known as IRES-transacting factors (ITAFs). MATR3 is an RBP part of the inner nuclear matrix implicated in RNA stability and splicing processes. MATR3 is also a Rev-cofactor, and binds the 5'UTR of HIV-1 vRNA. This study evaluated if MATR3 acts as an ITAF for the HIV-1 IRES.
Materials and Methods: A bicistronic vector, dl HIV-1 IRES, harboring the 5'UTR region of HIV-1 vRNA in the intergenic region was transfected in HEK293T cells with varying concentrations of plasmids encoding MATR3 and Rev. An siRNA targeting MATR3 encoding mRNA was used to evaluate the impact of endogenous MATR3 on IRES activity.
Results: The overexpression of MATR3 stimulates, while its knockdown decreases HIV-1 IRES activity. The overexpression of Rev did not affect the HIV-1 IRES activity. However, in the presence of Rev, the stimulatory effect of MATR3 is increased.
Conclusion: MATR3 is a stimulatory ITAF for the HIV-IRES activity.
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6. Genome structure and transcriptional regulation of a horizontally acquired region in yeast. Andrés Romero[1,2](aab.romero.q@gmail.com), Matteo De Chiara[3], Eduardo I. Kessi-Pérez[4], Gianni Liti[3], Francisco Salinas[1,2]. [1]Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile. [2]ANID-Millennium Science Initiative-Millennium Institute for Integrative Biology (iBio), Santiago, Chile. [3]CNRS, INSERM, IRCAN, Université Côte d'Azur, Nice, France. 4. Centro de Estudio en Ciencia y Tecnología de los Alimentos (CECTA), Universidad de Santiago de Chile (USACH), Santiago, Chile.

In *Saccharomyces cerevisiae* pangenome a DNA segment known as region B has been acquired horizontally from a distant yeast species. This region (~17 Kb) encodes for 5 genes whose contribution to yeast niche-specific adaptation has not been determined. Here, we demonstrated that region B is involved in oxidative stress response. Initially, we analyzed the genome structure of region B in 142 strains, identifying 10 variants that maintain a circular continuity. Then, we characterized the transcriptional activity of each gene within region B using a fluorescent reporter and utilizing five different genetic backgrounds. Our results indicated that gene expression within region B depends on genetic background. Region regulation was also assessed, observing that its expression is regulated by transcription factors encoded within this region and genes belonging to the core genome (*YEF1*, *DSD1*, and *RKM5*). Finally, using a collection of yeast strains including a strain with region B deletion, we demonstrated that region B is important for growth under H_2O_2, suggesting an active role of this region in oxidative stress response. Altogether, our results show the complex regulation of horizontally acquired genes into the host genome, increasing phenotypic plasticity and expanding yeast adaptation.
Supported by: ANID-FONDECYT 1210955 and ANID-PhD scholarship 21210525.

7. Distinct Inner Mitochondrial Membrane Morphology in Oxidative and Glycolytic Skeletal Muscle. Isidora Molina-Riquelme (iemolina@uc.cl)[1], José Díaz-Maldonado[1], Verónica Eisner[1]. School of Biological Sciences, Pontificia Universidad Católica de Chile.

Introduction: Skeletal muscle can be classified as oxidative and glycolytic based on its primary ATP production pathway. Classically, this difference is linked to mitochondrial content. However, other factors may influence the preference for a type of metabolism. Cristae morphology, where oxidative phosphorylation occurs, relies on the activity of the MICOS complex, OPA-1 and ATP synthase. We hypothesised that the metabolic differences in muscle types are supported by cristae morphology.
Materials and Methods: We dissected soleus (oxidative), *extensor digitorum longus* (EDL) and *flexor digitorum brevis* (FDB) (glycolytic) from three-month-old C57BL6/J mice. For transmission electron microscopy, each muscle was fixed in 2,5% glutaraldehyde and stained. Data were acquired at the TALOS 200kV microscope. Whole muscle lysates were used for qPCR and WB. Isolate mitochondria were used for respirometry.
Results: Cristae analysis showed greater cristae abundance in soleus compared to EDL and FDB, and a higher number of cristae per 100 nm. We found no differences in cristae width or junction. Also, qPCR showed higher OPA-1 transcripts abundance in soleus than in EDL. Mic60 transcripts levels were comparable between soleus and EDL. OPA-1 protein levels were higher in oxidative muscle, while Mic19 levels were higher in soleus and FDB than in EDL. Mitochondria respiratory complex assembly factors COX18 and SFNX4 levels were similar across muscles. Respirometry of isolated mitochondria showed lower pyruvate/malate-driven respiration and maximal respiratory capacity in FDB.

Discussion: This data suggests that cristae morphology differences between oxidative and glycolytic muscle, are not solely by cristae-shaping proteins. Differences in complex I-driven respiration in isolated mitochondria suggest muscle-dependent regulatory mechanisms.
Acknowledgements: ANID Ph.D. fellowship 21201041 to IM-R, FONDECYT grant 1191770 to VE.

8. The long intergenic non-coding RNA *698* is dysregulated during cardiomyocyte differentiation of iPSCs from Down Syndrome individuals. Francisco Sigcho[1,2,3](panchosigcho@gmail.com), Wladimir Corrales[3,4], Leslye Venegas[1,2], Sebastián Leiva[1,2,3], Alonso Torres Ricke[1], Rodrigo Aguilar[5], Vinicius Maracaja-Coutinho[2,3,6], Valentina Parra[1,2,6]. [1]Laboratorio de Diferenciación Celular y Metabolismo, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile. [2]Advanced Center of Chronic Diseases (ACCDiS), Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile. [3]Centro de Modelamiento Molecular, Biofísica y Bioinformática (CM2B2), Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile. [4]Laboratorio de Neuroplasticidad y Neurogenética, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile. [5]Institute of Biomedical Sciences, Faculty of Medicine and Faculty of Life Sciences, Universidad Andres Bello, Santiago, Chile. [6]Systems Biology Center for the Study of Extremophile Communities from Mining Tailings (SYSTEMIX), O'Higgins University, Rancagua, Chile

Introduction: Down syndrome (DS) is a genetic condition that disrupts cellular processes, including cardiogenesis, increasing the risk of congenital heart diseases. Cardiogenesis in induced pluripotent stem cells (iPSCs) from DS individuals (3S) is impaired compared to non-trisomic individuals (2S). Long non-coding RNAs (lncRNAs) are known regulators of cardiogenesis, but their role in DS is unclear. We hypothesized that “lncRNAs associated with cardiogenesis are differentially expressed (DE) during cardiomyocyte differentiation of 3S-iPSCs.”
Materials and Methods: A meta-analysis of public transcriptome datasets identified DE lncRNAs (p-value < 0.05, FC > 1.5) between 3S and 2S iPSCs. Transcription factors (TFs), cardiac markers (CM), and lncRNAs were measured during 2S and 3S-iPSC differentiation. Cardiac differentiation efficiency was assessed using immunofluorescence. Co-expression analysis using CEMITOOL explored potential lncRNA mechanisms.
Results: The analysis revealed four DE lncRNAs (*MIAT*, *B3GALT5-AS1*, *LINC00205*, *LINC00698*) overexpressed in 3S-iPSCs. Cardiac markers (*TNNT2*, *NX2-5*, *MYH6*, *GATA4*) were downregulated in 3S-derived cardiomyocytes, linked to early developmental stage dysregulation, indicated by downregulation of mesoderm specification TFs (*BRACHYURI*, *MESP*) and cardiac progenitor induction (*ISL1*, *MEF2C*). Pluripotency markers (*SOX2*, *NANOG*, *POU5F*) persisted longer in 3S conditions. Cardiomyocyte differentiation efficiency

in 3S-iPSCs was significantly lower than in 2S-iPSCs. *LINC00698* was upregulated during mesoderm specification and decreased in subsequent stages in 2S-iPSCs but remained elevated in 3S differentiation, suggesting a role in early cardiogenesis dysregulation. Co-expression analysis indicated that *LINC00698* is negatively correlated with cardiac development genes (BMP-family, TBX-family, *MYH6*, and *MYH7*) and positively correlated with pluripotency markers (*DPPA4*, *NANOG*, and *L1TD1*).
Discussion: Cardiomyocyte differentiation in 3S-iPSCs is impaired compared to 2S-iPSCs due to early TF downregulation. LINC00698 plays a crucial role in mesoderm specification, and its abnormal regulation in 3S conditions likely disrupts differentiation. Further studies are needed to validate the associations of LINC00698 with other cardiomyocyte differentiation genes.
Funding: This project is funded by ANID FONDECYT 1230195 (VP), 1211731 (VM-C), Anillo SYSTEMIX ACT210004 (VP, VM-C), FONDAP 15130011 (VP, VM-C) and ANID PhD scholarship 21210841 (FS); Universidad de Chile grants Enlace FONDECYT I0230/2020 (VM-C) and Apoyo a la Infraestructura para la Investigación INFRA-021/01/2019 (VM-C) and INFRA037/2023 (VP).

Oral session 4

1. Effect of Sortin2 as a Substance Enhancing Salt Stress Resistance in *Arabidopsis thaliana*.
Francisco P. Malmborg (Francisco.pavez.m@ug.uchile.cl), Lorena Norambuena. Plant Molecular Biology Centre, Department of Biology Faculty of Science, Universidad de Chile.

Chile is experiencing a loss of regular rainfall patterns due to climate change, leading to prolonged drought periods, where crops face stress from increased soil salinity. In this context, the scientific community is seeking for strategies to mitigate the effects of salt stress due to NaCl. The advantage of using chemical biology as mitigation strategy appears promising and farmer friendly. Thus, we have tested bioactive compounds with the aim of increase the plants salt tolerance. Our study focused on Sortin2, a synthetic compound that enhances endocytic trafficking; cellular process that has been linked to salt stress response. Based on this, we aimed to test whether this compound protect *Arabidopsis thaliana* plants from the salt stress. Then we challenge Arabidopsis seedlings with NaCl and measure physiologic parameter to evaluate salt stress effect. On the other hand, the effect of Sortin2 on several hormones pathways was evaluated.
Sortin2 increased the tolerance to the toxicity of Na^+ stress improving physiological parameters. From a hormonal perspective, using reporter lines, we observed that Sortin2 induces hormonal changes similar to those caused by NaCl treatment, increasing responses to abscisic acid, brassinosteroids, and jasmonate, while decreasing the response to cytokinins. Overall, our findings demonstrate that Sortin2 is a promising compound for enhancing salt stress tolerance in plants.
Acknowledgment: FONDECYT 1211311; ACT192073.

2. Effect of Lipoic Acid Application on Leaf Senescence and Tomato Fruits. Felipe Uribe-Cárdenas[1] (felipe.uribe.c@ug.uchile.cl), Reinaldo Campos[2], Michael Handford[1]. [1]Universidad de Chile, Centro de Biología Molecular Vegetal, Departamento de Biología, Facultad de Ciencias, Santiago, Chile. [2]Universidad de Chile, Centro de Estudios Postcosecha, Departamento de Producción Agrícola, Facultad de Ciencias Agronómicas, Santiago, Chile.

Introduction: Plants face adverse conditions such as drought, which increases the unwanted production of oxidative molecules. Therefore, plants produce antioxidant compounds such as lipoic acid (LA) to combat the damaging effects of such molecules. The synthesis of LA is catalyzed by lipoyl synthase and uses the precursor S-adenosylmethionine (SAM), a molecule also required for ethylene synthesis, a key phytohormone in leaf senescence and fruit maturation in climacteric plants. This study proposes that there is an antagonistic regulation between the synthesis of LA and ethylene due to competition for SAM. Therefore, the effects of altering the ethylene and LA synthesis pathways on senescence, fruit maturation, and the antioxidant response are being determined by exogenously applying LA to tomato plants.
Materials and Methods: Leaves and fruits of *Solanum lycopersicum* plants were sprayed with 200-500 nM LA. Senescent leaves and fruits at different stages of ripening were analyzed by evaluating transcript levels of genes in the LA and ethylene synthesis and effector pathways using RT-qPCR, and levels of lipoylated proteins were determined by Western Blot.
Results: It was observed that the application of LA delays the development of chlorosis of older leaves of the plants (as an indicator of leaf senescence), which correlates with higher transcript levels of LA pathway genes, and a greater content of lipoylated proteins. In fruits, a similar effect in terms of protein lipoylation was also observed after application of LA. In both organs, such molecular effects were accentuated at the higher of the two LA concentrations.
Discussion: These results suggest that the application of LA influences the leaf senescence process and in different states of fruit maturation. This is relevant because the application of LA in tomato plants could increase tolerance to oxidative stress and delay leaf senescence.
Acknowledgments: Fondecyt N°1231417 (MH) and ANID Doctoral Scholarship N°21210768 (FU).

3. Exploring the role of pectin metabolism in nitrate-induced cell expansion in *Arabidopsis thaliana* cotyledons. Valentina Nunez-Pascual[1,2] (v.nunezpascual@gmail.com), Eleodoro Riveras[1,2], Christian Silva-Sanzana[2,3], Tomas Moyano[1,2], Francisca Blanco-Herrera[2,3], Susana Saez-Aguayo[3], Ariel Orellana[3], Sarah Robinson[4] and Rodrigo A. Gutiérrez[1,2]. [1]Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile; Millennium Institute Center for Genome Regulation; Institute

of Ecology and Biodiversity. [2]Millennium Institute of Integrative Biology. [3]Centro de Biotecnología Vegetal, Facultad de Ciencias de la Vida, Universidad Andrés Bello. [4]Sainsbury Laboratory of Cambridge University.

Introduction: Nitrate is one of the most important nitrogen sources for plants. Besides its nutritional role, nitrate acts in plants as a signal molecule that regulates several processes including growth and development. In cotyledons and true leaves, nitrate promotes growth by inducing cell expansion. Bioinformatics analysis of transcriptome data indicated cell wall organization is a relevant process among differentially expressed genes by nitrate. However, there is little or no information about the influence of nitrate in cell wall composition and properties. Here, we explored the interaction between nitrate-induced growth and cell wall metabolism with emphasis on the role of the pectin matrix.
Methods: Using *Arabidopsis thaliana* cotyledons grown under contrasting nitrate conditions, we characterized cell wall monosaccharide composition with HPLC and pectin changes with colorimetric assays and confocal microscopy. We also evaluated the impact of nitrate on pectin-modifying enzyme activity. Finally, we evaluated changes in cell wall elasticity using atomic force microscopy (AFM) and automatic confocal microextensometry (ACME).
Results: We found nitrate availability affects pectin methylesterification status and pectin methylesterase activity during expansion. Additionally, we observed changes in cell wall biomechanical properties in response to nitrate.
Discussion: Together, our results support a model where pectin metabolism could be involved in nitrate-induced cell growth.
Acknowledgements: Millennium Institute for Integrative Biology (ICN17_022), Center for Genome Regulation (ICN2021_044), ANID-FONDECYT 1220594, Beca Doctorado Nacional ANID.

4. Shedding light into the structural dynamics of the human voltage-gated proton channel by using transition metal ion FRET and genetic code expansion. Emerson M. Carmona (emerscr@uw.edu), William N. Zagotta, Sharona E. Gordon. Department of Physiology and Biophysics, University of Washington, Seattle, WA 98195.

Introduction: The human voltage-gated proton channel (hH_v1) is a membrane protein with a selective permeation pathway for protons. Interestingly, hH_v1 does not contain a pore domain, and its voltage sensor domain is activated by various stimuli. Structural models of hH_v1 have been reported, but the molecular mechanisms explaining the hH_v1 function are still poorly understood.
Materials and Methods: We will study the conformational dynamics of hH_v1 by measuring short-distance distributions of the protein through transition metal ion FRET (tmFRET). As the first step of this project, we incorporated the fluorescent noncanonical amino acid acridon-2-yl-alanine (Acd) in H_v1 using genetic code expansion in E. coli as a tmFRET donor.

Results: We studied the expression, stability, function, and fluorescence of the Cysless version of hH_v1 with Acd incorporated at 14 positions along the protein sequence. The level of expression and the presence of truncated products varied across positions. Moreover, differences in the fluorescence-detection size exclusion chromatography peaks were observed between constructs. We successfully purified functional hH_v1-Acd for 12 of these constructs. Changes in the fluorescence spectrum and lifetimes of hH_v1 with Acd incorporated in certain positions were observed when the protein was transferred from a detergent micelle to a lipid bilayer.
Discussion: The differential spectral properties of Acd demonstrate that we incorporated site-specifically this noncanonical amino acid co-translationally. Currently, we are inserting cysteine residues in these hH_v1-Acd constructs to insert the transition metal ion using cysteine-reactive chelates as the tmFRET acceptor.
Acknowledgment: EMC is a Pew Latin American Fellow.

Sponsored by: Dr. Victor Castro.

5. Identification of key residues in the evolution of substrate specificity in the bacterial ATP-dependent vitamin kinase enzymes. Nicolás Fuentes-Ugarte (nicolas.fuentes@ug.uchile.cl), Myriam Pérez, Isaac Cortés-Rubilar, Isabel Asela-Montes, Belén Valderrama-Plaza, Nikolas Knoop-Siegel, Gabriel Vallejos-Baccelliere, Victoria Guixé & Víctor Castro-Fernández. Laboratorio de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Chile, Santiago, Chile.

In bacteria, vitamin B1 (thiamine) biosynthesis requires the phosphorylation of 4-amino-2-hydroxymethyl-5-methylpyrimidine (HMP) to HMP-PP, with HMP-P as an intermediate. Two homologous enzymes can phosphorylate HMP to HMP-P: 1) a bifunctional pyridoxal kinase ThiD2-ccPLK/HMPK, that also phosphorylate pyridoxal (PL) and 2) the ThiD-HMPPK, which also phosphorylates HMP-P to HMP-PP. This latter phosphorylation is unique within the ATP-dependent vitamin kinase family because it involves the phosphorylation of a methyl-phosphate group, in contrast with the typical primary alcohol group phosphorylation performed by the other kinases. The described reaction mechanism for enzymes of this family had a catalytic base (Asp or Cys) to activate the alcohol and a positive charge (Arg or Lys) to stabilize the transition complex.
Phylogenetic analyses indicate that ThiD2-ccPLK/HMPK enzymes diverged from ThiD-HMPPKs, suggesting the loss of HMP-P kinase activity and the emergence of PLK activity as an evolutionary novelty in the ThiD2-ccPLK/HMPK group. Crystal structures of an ancestral ThiD-HMPPK from *Enterobacterales* (ancEnHMPPK) show that K111 residue may stabilize the transition state, while the C213, reported as a catalytic base, is too far from the phosphoryl acceptor group of HMP-P to be catalytic. Interestingly, residues H179 and T211 appear to be relevant for HMP-P phosphorylation.

To investigate the evolutionary transition in the substrate specificity, ancestral sequences were reconstructed, expressed, purified, and characterized, showing that the last common ancestor of ThiD-HMPPK phosphorylates HMP and HMP-P. In contrast, the intermediate ancestor (ancC) to ThiD2-ccPLK/HMPK gained the ability to phosphorylate PL while the last common ancestor of ThiD2-ccPLK/HMPK optimized this capability.
Finally, to evaluate the roles of residues K111, H179, T211, and C213 in HMP-P phosphorylation, we performed site-directed mutagenesis on ancEnHMPPK. As expected, the mutants revealed the crucial importance of residues H179 and T211 only for HMP-P phosphorylation. In contrast, residue C213 is essential for both HMP and HMP-P phosphorylation.
Acknowledgment: FONDECYT 1221667.

6. Clade-wide proteome analysis shows widespread non-canonical DCR proteins in Fungi. Lorena Melet[1,2,3], Jonathan Canan[1,2], Pablo Villalobos[4], Víctor Castro[4], Nathan R. Johnson[1,2], Elena A. Vidal[1,2]. [1]Centro de Genómica y Bioinformática, Universidad Mayor, Chile. [2]ANID-Millennium Science Initiative Program-Millennium Institute for Integrative Biology (iBio), Chile. [3]Programa de Doctorado en Genómica Integrativa, Universidad Mayor, Chile. [4] Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Chile.

Introduction: Dicer (DCR) is a class-III ribonuclease with a fundamental role in the synthesis of small RNAs and the regulation of gene expression. DCRs are found in many eukaryotic organisms, including fungi, where they are essential for defense against viruses, transposon regulation, and development. To date, fungal DCRs have mainly been identified in well-studied phyla: *Ascomycetes*, *Basidiomycetes*, and *Chytridiomycetes*. However, many fungal species still lack described DCRs, and there is no clear understanding of the structural domains present in fungal DCRs.
Materials and Methods: We determined putative DCR proteins from proteomes of 1,423 species spanning 9 fungal phyla. Aminoacidic sequences were scanned with HMM matrices representing the main domains described for DCR proteins to obtain the putative set of fungal DCRs. Protein models of the predicted DCRs were generated with AlphaFold to determine their structural similarity and phylogenetic trees and ancestral reconstructions were carried-out to gain insights into the evolutionary story of the proteins. Finally, for a selected set of DCRs with non-canonical structures, protein-RNA docking simulations were performed to infer their possible functionality.
Results: We found that only a small fraction of the proteins contains all the canonical domains described for plant and animal DCRs. Many fungi lack a PAZ domain, which serves to bind and recognize the 3' terminal overhangs of the pre-miRNA and influences the length of the small RNAs produced. Phylogenetic analyses suggest that this domain may have evolved in a common ancestor of fungi. However, despite the absence of the PAZ domain these non-canonical DCRs still fold in a similar manner to canonical DCR proteins, and *in silico* evidence indicates their ability to bind double strand RNA molecules.

Discussion: This work presents the first comprehensive catalog of DCR proteins in fungi, highlighting their relevant features and putative functional roles in sRNA biogenesis.
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7. CryoEM reveals the oligomeric diversity of the postsynaptic protein gephyrin: challenging the current model of postsynaptic density organization. Diego Ortiz-López[1] (diego.ortiz-lopez@uni-wuerzburg.de**),** Tamsamqa Tafara Hove[1], Benjamin Campbell[2],Shiva K Tyagarajan[2], Hans Maric[1] and Hermann Schindelin[1].(1)Rudolf Virchow Center for Experimental Biomedicine University of Würzburg, Würzburg Germany. (2) Department of Biochemistry, University of Zürich, Zürich, Switzerland

At inhibitory synapses, the position and distribution of GABA and glycine receptors depend on the scaffolding protein gephyrin, which forms subcellular structures known as postsynaptic densities (PSDs). How gephyrin modulates this process remains poorly understood due to a lack of structural insights into the architecture of the full-length protein. Here, we introduce a new approach using Designed Ankyrin Repeat Proteins (DARPINs) to obtain stable complexes for structural characterization. By analyzing our samples with cryo-electron microscopy (CryoEM), size-exclusion chromatography coupled to multi-angle light scattering (SEC-MALS), and other biochemical techniques, we demonstrate that gephyrin scaffolds are organized into different oligomers and conformations. These findings suggest a potential semi-flexible PSD network structure, arguing against a stable, fully organized structure.
Acknowledgment: Deutsche Forschungsgemeinschaft (DFG) 232550447.

8. Characterization of the conformational changes in the Crimean-Congo Hemorrhagic Fever virus spikes. Esteban Rodríguez Rivera[1] (erodriguez@cienciavida.org), Gianina Arata[1], Eduardo A. Bignon[1], Nicole D. Tischler[1,2]. [1]Laboratorio de Virología Molecular, Centro Ciencia & Vida, Fundación Ciencia & Vida, Santiago, Chile. [2]Escuela de Bioquímica, Facultad de Medicina y Ciencia, Universidad San Sebastián, Santiago, Chile.

Introduction: Nairoviruses are tick-borne viruses that includes Crimean-Congo Hemorrhagic fever virus (CCHFV), which can infect mammals including humans, where it can cause up to 30% of lethality. Thus, the WHO cataloged CCHFV as a priority agent in infectious diseases. Nairoviruses include a lipid envelope projecting Gn/Gc glycoprotein spikes, with Gc having a class II fusion protein fold. This type of fusion proteins is generally activated by acidic pH, where it is commonly rearranged into a highly stable post – fusion homotrimer. The spikes induce virus cell entry by receptor interaction and subsequent endocytosis, where it takes the virus-cell membrane fusion. Because the spikes are surface exposed, they are the main targets of neutralizing antibody responses, and hence represent

attractive antigens to induce immunity. Here, we analyze if the membrane fusion of CCHFV is triggered by acidification, and next we describe the Gc oligomeric changes.
Materials and methods: We obtain virus-like particles (VLPs) of CCHFV through transfection of HEK293FT cells. We analyzed their reactivity with anti-Gc monoclonal antibodies, through flow virometry. Next, we analyze the oligomeric structure of the spikes by BN-PAGE, where we expose them to different conditions. Through liposome-coflotation assays we evaluate if CCHFV VLPs interacts with artificial endosomal-membranes (liposomes). To study if Gc forms the canonical homotrimeric highly stable post – fusion conformation, we used sucrose sedimentation and BN-PAGE.
Results: We demonstrate CCHFV VLPs are pleomorphic with ≥ 160 nm diameter. We show that Gc forms different complexes depending on the pH. Lastly, the pH-induced conformation of Gc does not reassemble a stable post – fusion homotrimer.
Discussion: We demonstrate that Gc changes its oligomerization upon acidification. However, additional factors are required to induce the post – fusion form.
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POSTERS ABSTRACTS

1. Alginate Oligosaccharides Protect Gastric Epithelial Cells against Oxidative Stress Damage through Induction of the Nrf2 Pathway. Samantha Acevedo[1](samantha.acevedo@ce.ucn.cl), Alejandra A. Covarrubias [2,3], Paola Haeger [4,5,6], Floria Pancetti [2,6,7], Fadia Tala [6,7,8,9] and Erwin de la Fuente-Ortega [1,6,7]. [1]Laboratorio de Estrés Celular y Enfermedades Crónicas no Transmisibles, Universidad Católica del Norte, Coquimbo 1781421, Chile. [2]Laboratorio de Neurotoxicología Ambiental, Departamento de Ciencias Biomédicas, Facultad de Medicina, Universidad Católica del Norte, Coquimbo 1781421, Chile. [3]Facultad de Ciencias Agropecuarias, Universidad del Alba, La Serena 1700000, Chile. [4] Laboratorio de Neurobiología de la Conducta, Departamento de Ciencias Biomédicas, Facultad de Medicina, Universidad Católica del Norte, Coquimbo 1781421, Chile. [5]Millennium Nucleus of Neuroepigenetics and Plasticity (EpiNeuro), Santiago 8370186, Chile. [6]Núcleo de Investigación en Prevención y Tratamiento de Enfermedades Crónicas no Transmisibles (NiPTEC), Universidad Católica del Norte, Coquimbo 1781421, Chile. [7]Centro de Investigación y Desarrollo Tecnológico en Algas y Otros Recursos Biológicos (CIDTA), Facultad de Ciencias del Mar, Universidad Católica del Norte, Coquimbo 1781421, Chile. [8]Departamento de Biología Marina, Facultad de Ciencias del Mar, Universidad Católica del Norte, Coquimbo 1781421, Chile. [9]Instituto Milenio en Socio-Ecología Costera, SECOS, Santiago 7550000, Chile.

Introduction: Gastric diseases represent a significant global public health challenge, characterized by molecular dysregulation in redox homeostasis and heightened oxidative stress. Although prior preclinical studies have demonstrated the cytoprotective antioxidant effects of alginate oligosaccharides (AOSs) through the Nrf2 pathway, whether such mechanisms apply to gastric diseases remains unclear.
Materials and Methods: The GES-1 gastric cell line was exposed to hydrogen peroxide (H_2O_2) as a damage model to investigate the impact of AOS on cell viability, ROS levels, and its associated mechanisms by RT-qPCR, Western blot, and Immunofluorescence.
Results: Our results revealed that pre-incubation with AOS for either 4 h or 24 h significantly improved the viability of GES-1 cells exposed to H_2O_2. In addition, AOS reduced the intracellular ROS levels, activating the Nrf2 signaling pathway, with increased Nrf2 protein and mRNA expression and a significant upregulation of the target genes HO-1 and NQO1. The activation of Nrf2 was correlated with decreased Keap1 protein expression and an increased level of the autophagy protein p62/SQSTM1.
Discussion: These experiments suggest the activation of Nrf2 through a noncanonical pathway. This indicates that AOS is a potential treatment for protecting gastric epithelial cells from oxidative stress by activating the p62/SQSTM1-Keap1-Nrf2 axis and laying the foundation for future investigations about its specific therapeutic mechanisms.

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2. Probing the stability of protein secondary structure by electronic structure calculations of infinite polyalanine chains. Francisco Adasme-Carreño[1,2], Camila Muñoz-Gutiérrez[1], Joel Ireta[3]. [1]Centro de Investigación de Estudios Avanzados del Maule (CIEAM), Vicerrectoría de Investigación y Postgrado, Universidad Católica del Maule, Talca 3480112, Chile. [2]Laboratorio de Bioinformática y Química Computacional (LBQC), Departamento de Medicina Traslacional, Facultad de Medicina, Universidad Católica del Maule, Talca 3480112, Chile. [3]Departamento de Química, División de Ciencias Básicas e Ingeniería, Universidad Autónoma Metropolitana - Iztapalapa, A.P. 55-534, Ciudad de México 09340, México.

Introduction: Deciphering the forces driving a protein to adopt the native conformation is crucial to understand the folding process. Helices and β sheets are associated to low-energy regions of the conformational space of proteins. We have previously calculated the potential energy surface (PES) of an infinite polyalanine chain using density functional theory (DFT), which accounts for intra-chain H-bonds and cooperativity. Well-defined energy basins in the PES were connected to regular secondary structures. Here we have studied how inter-chain H-bonds stabilize β sheets and evaluate the intrinsic structural preferences of proteinogenic amino acids.
Materials and methods: DFT calculations of bidimensional periodic polyalanine and host-guest systems for non-charged amino acids at the geometries of the energy minima were carried out using PBE+TS and plane waves with VASP in vacuum at 0 K.
Results: Extended conformations (γ helix, PPII, and β sheet) are significantly more stable (4-5 kcal/mol) upon formation of inter-chain H-bonds, and infinite β sheet appears as the most stable conformation compared to α helix (the most abundant subtype). Host-guest models showed that non-alanine amino acids prefer helical over extended conformations, however the order of preference varies among the amino acids. Solvation does not change the ordering but reduces the energy gap between helical and extended conformations.
Discussion: The greater stability of the infinite β sheet may be linked to the formation of amyloid aggregates. Side chain does not seem to affect the overall stability of the secondary structures. These findings will help to further our understanding of protein structure and folding.
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3. Identification of relevant regions in the MALAT1 transcript and identification of its binding sites in a cancer cell genome. Constanza Mardones[1], Cristopher Fierro[1], Hugo Sepulveda[1], Roberto Munita[2], Rodrigo Aguilar[1] (rodrigo.aguilar@unab.cl). [1]Institute of Biomedical Sciences, Universidad Andres Bello. [2]Universidad de Chile.

Introduction: MALAT1 is a long non-coding RNA involved in gene regulation. It is associated with proliferation, migration, and survival in several cancers. Its structure includes several helices, a triple helix, G-quadruplex motifs and interacts with RNA-binding proteins. However, which modules within the RNA are relevant for function is still unknown. To address this, we embarked on identifying and characterizing relevant regions within the MALAT1 sequence. Also, we determined MALAT1 binding sites to the genome, which may be involved in gene expression regulation related to cancer proliferation and progression.
Materials and Methods: We performed *in silico* analyses using the UCSC Genome Browser, focusing on the secondary structural model of the human MALAT1. We looked for evolutionary conserved sites, guanine-rich regions, and protein-binding sites from eCLIP analyses. Concurrently, we conducted an *in silico* analysis of published CHART data, using computational tools, generating BED files, which were processed through Bedtools to filter and select data subsets.
Results: We found high degree of evolutionary conservation in the MALAT1 transcript, especially in speckle-targeting E and M regions. We found G-quadruplexes and binding of RBP proteins at a few conserved sites. CHART-seq data indicated that MALAT1 is enriched at promoters, potentially regulating important genes in cancer.
Discussion: Our findings provide a comprehensive map of putative key regions in the MALAT1 transcript and its binding sites in the genome of cancer cells. The data will offer new perspectives for therapeutic strategies targeting lncRNAs in cancer treatment.
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4. Kinetic and structural characterization of the pyruvate kinase from the class II methanogenic archaeon *Methanosarcina thermophila*. Antonia Alarcón-Saavedra (antonia.alarcon.s@ug.uchile.cl), Gonzalo Quiñones-Pérez, Sebastián M. Muñoz, Ignacio Aravena-Valenzuela, Víctor Castro-Fernández, Victoria Guixé. Laboratorio de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Chile. Santiago, Chile.
Methanogenic archaea are anaerobic organisms whose energy generation is based on methane production and can be classified into two classes based on their methanogenic pathways. Class I methanogens use only hydrogenotrophic pathway, while class II methanogens besides hydrogenotrophic pathway, use acetoclastic and methylotrophic pathways. When substrates for methanogenesis are depleted, they degrade glycogen through the glycolysis pathway, where the reaction catalyzed by the enzyme pyruvate kinase (PK) plays an essential role. This enzyme catalyzes the last step of glycolysis, allowing the transfer of a phosphoryl group from phosphoenolpyruvate (PEP) to Mg-ADP, generating pyruvate and ATP being highly regulated in all the organisms studied. In eukarya, PKs are

activated by fructose-1,6-bisphophate (FBP). In some bacteria, PKs are activated by FBP and AMP. Nonetheless, information regarding the allosteric regulation of archaeal PK is scarce. It has been reported that AMP is an activator in some PKs from *Methanococcales* (class I methanogens) and *Halobacteriales* orders. At the same time, 3-phosphoglycerate (3PG) is an activator of PKs from *Crenarchaeota* phylum.
To understand if PKs from methanogenic archaea from other orders besides *Methanococcales* present allosteric regulation, we performed the kinetic and structural characterization of the pyruvate kinase enzyme of *Methanosarcina thermophila* (MthPK) of the phylum *Methanosarcinales* (class II methanogens). Using recombinant expression, MthPK was purified and kinetic parameters for its corresponding substrates were determined. Through SEC-MALS, we determined that the protein is a tetramer in solution. We also obtained a crystallographic structure at 3 Å resolution and proposed a tetrameric model based on the asymmetric unit using PISA prediction. Evaluating of potential allosteric effectors showed that MthPK is not activated by AMP, unlike some PKs form *Methanococcales*. These results contribute to a better understanding of the regulation of the glycolytic pathway in methanogenic archaea of class II and to the structural of archaeal PKs.
FONDECYT 1221667, 1231263.

5. Characterization of Dye Sensitized Solar Cells with Antarctic bacteria pigments.
Isabel Alarcón-Fica (isalarcon2016@udec.cl)[1], Jose Martinez-Oyanedel[1], Paulraj Manidural[2], Bayron Cerda[2]. [1]Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción. [2]Departamento de Física, Facultad de Ciencias Físicas y Matemáticas, Universidad de Concepción.

Dye-sensitized solar cells (DSSC) have become an alternative to silicon cells for obtaining energy through light, due to their low manufacturing cost and relative ease of location for their operation, because can be manufactured with flexible materials and transparent surfaces.
These cells are composed of a photo electrode able of capturing light energy through the pigment attached to a TiO2 layer, which acts as a semiconductor, and has a Pt counter electrode and an electrolyte that allows the continuous transfer of electrons between the light excited pigment and the counter electrode.
To evaluate the stability and efficiency of the DSSCs, Electrochemical Impedance Spectroscopy (EIS) was used to study the efficiency in charge transfer or ion diffusion. Curve IV provides data on the behavior of the cell when applying an increasing voltage, thus giving a clear idea of the capacities in its conversion of light energy to electrical energy.
In the present work, from strains of colored Antarctic bacteria, we obtained their pigments and characterized in their spectroscopic properties with UV-vis and Fluorescence spectra. We assembled DSSC cells and evaluated it by measuring their current-voltage curve with a PET solar simulator and their conductivity characteristics were measured using the Electrochemical Impedance Spectroscopy technique.

The results obtained show that the cell sensitized with the pigment of the Antarctic bacteria P29M1-25 is the one with the highest efficiency of 0.050%, and that this efficiency increases to 0.1% when we use APTMS to fix the pigment. For the EIS results, the P29M1-25 pigment, although it presents a high resistance due to its problem in recovering the redox state by the electrolyte, presents a parallel capacitance that compensates for this effect, allowing a relatively good efficiency.
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6. Characterization of Sec61 post-translationally translocon by Mass Photometry. Hilda M. Alfaro-Valdés[1,2] (hilda.m.alfaro.v@gmail.com), Nathalie Casanova-Morales[3], John William Young[4], Philipp Kukura[4] and Christian A.M. Wilson[1]. [1]Faculty of science, University of Valparaíso, Valparaíso, Chile. [2]Laboratory of Single Molecule Biochemistry and Mechanobiology, Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile. [3]Faculty of Liberal Arts, Adolfo Ibáñez University, Santiago, Chile. [4]Physical and Theoretical Chemistry Laboratory, Department of Chemistry, University of Oxford, Oxford, UK.

The post-translationally translocon from *Saccharomyces cerevisiae* is composed by the Sec61 channel, Sec62, Sec63, Sec71 and Sec72 proteins. Sec61 translocon is essential for protein translocation across the endoplasmic reticulum (ER). BiP is a chaperone of the ER that allows the translocation of proteins through the interaction with J Domain of Sec63. However, the details of the interaction between BiP and Sec63 remain unclear. Largely due to the dynamic nature of these interactions and limitations in experimental methodologies. This study explores 3 strategies experimental for purifying and reconstituting the Sec61 translocon using Peptidisc (small peptides that bind hydrophobic patches), aiming to improve the stability and solubility of the Sec61 Translocon. We found that the Sec61 translocon can be stably reconstituted with peptidiscs added to microsomes mixed with detergent during affinity column purification. The Sec61 translocon's was assessment by mass photometry (MP). MP is a technique that allows determining the mass of different species in a sample by quantifying the scattering of light as it interacts with proteins in solution. We identified that the Sec61 translocon was found in the fraction near the center of a linear sucrose gradient. ATP and ATPγS treatments demonstrated the functional integrity of the complex.
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7. The reduction of glutathione levels in the bacteria pathogen *Enterococcus faecalis* trigger metabolic and transcriptional global adjustments under iron exposure. Víctor Aliaga-Tobar[1,2,3] (victor.aliaga.tobar@gmail.com), Sebastián Mendoza[4], Gabriel Gálvez[2,3], Jaime Ortega[2,3], Fernanda Fredericksen[2,3], Jorge Torres[2,3], Sebastián Gómez[2,3], Javiera Pino[2,3], Valentina Parra[6,7], Felipe Arenas[8], Alejandro Maass[5], Anne Siegel[9], Mauricio

González[10,11], Mauricio Latorre[2,3,5]. [1] Centro de Genómica y Bioinformática, Facultad de Ciencias, Ingeniería y Tecnología, Universidad Mayor, Santiago, Chile. [2] Laboratorio de Bioingeniería; Instituto de ciencias de la ingeniería; Universidad de O'Higgins, Libertador Bernardo O'Higgins 611, Rancagua, Chile. [3] Centro de biología de sistemas para el estudio de comunidades extremófilas de relaves mineros (SYSTEMIX), Universidad de O'Higgins, Libertador Bernardo O'Higgins 611, Rancagua, Chile. [4] Laboratorio de Bioinformática y Expresión Génica, INTA, Universidad de Chile, El Líbano 5524, Macul, Santiago, Chile. [5] Center for Mathematical Modeling, University of Chile, Santiago, Chile. [6] Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas & Farmacéuticas, Universidad de Chile, Santiago, Chile [7] Advanced Center for Chronic Diseases (ACCDiS), Facultad de Ciencias Químicas & Farmacéuticas & Facultad Medicina, Universidad de Chile, Santiago, Chile. [8] Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile (USACH), Santiago, Chile. [9] IRISA, Univ Rennes, Inria, CNRS, Rennes, France. [10]Bioinformática y Expresión Génica, Instituto de Nutrición y Tecnología de los Alimentos, Universidad de Chile, Santiago, Chile. [11]Center for Genome Regulation (Fondap 15090007), Universidad de Chile, Santiago, Chile.

Introduction: The reactive oxygen species (ROS) are toxic to cells, producing damage in cellular components and eventually cell death. In this sense, the tripeptide glutathione (GSH) has been described to be involved in ROS defense. Considering that i) *Enterococcus faecalis* is a pathogenic facultative anaerobe and common member of the gastro-intestinal tract microbiota that must deal with iron levels within the host and ii) that iron is associated with chemical reactions that produce ROS in bacteria intracellular environment, our interest fell in the response of this bacterium to increased levels of iron in a glutathione deficiency context.
Material and Methods: Using a mutant strain for Glutathione Synthetase (Δ*gsh*), we studied the response of *E. faecalis* under the increment of iron levels in a glutathione deficiency context, integrating transcriptional changes with a genome-scale metabolic model.
Results: The results showed that in glutathione deficiency, *E. faecalis* drastically reduces the intracellular levels of iron compared to wild type strain (WT). Indeed, the transcriptional change in Δ*gsh* strain assembles the counterpart of WT, reducing the expression of genes related to transcription and prioritizing genes related to basal metabolism. Posterior metabolites analysis in conjunction with differentially expressed genes used for a genome-scale metabolic model demonstrated that amino acids L-serine, L-aspartic acid, glycine, isoleucine, leucine and glycine decreased under iron excess in WT, not so in Δ*gsh* strain. In addition, the metabolic model also demonstrated that the Δ*gsh* strain prioritizes energy production in contrast with WT strain.
Discussion: Together, our results denoted a surprising metabolic flexibility of *E. faecalis* to deal with iron increase in a GSH decrease context, in which the increase of energy production appears to be required for iron efflux. In summary, the work provides relevant

insights in the response of pathogenic bacteria to iron increase in the context of glutathione deficiency.
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8. HnRNPK increases H6PD expression contributing to endoplasmic reticulum (ER) homeostasis in colorectal cancer cells. Karen Almendras (kalmendras2019@udec.cl), Alejandro Farías, Roxana Pincheira, Ariel Castro. Signal Transduction and Cancer Laboratory. Dept. Biochemistry and Molecular Biology. Universidad de Concepción

Introduction: hnRNPK is a multifunctional protein involved in various cellular processes, such as splicing, transcription, translation, and mRNA stabilization. It is overexpressed and associated with poor prognosis in colorectal cancer (CRC). RNA-seq analysis showed that hnRNPK downregulation increases the genes associated with the unfolded protein response (UPR) in lung cancer cells. Additionally, hnRNPK downregulation on cardiomyocytes increased the levels of ATF4, a marker of UPR. Altogether, these results suggest an association of hnRNPK with the UPR pathway. Analysis of RNA-seq, RIP-seq, and eCLIP-seq data show that hexose-6-phosphate dehydrogenase (H6PD) is a potential target of hnRNPK. H6PD, like glucose-6-phosphate dehydrogenase (G6PD, the rate-limiting enzyme of the pentose phosphate pathway (PPP), promotes the conversion of hexose phosphates at the endoplasmic reticulum (ER), generating NADPH. Previous studies showed that H6PD downregulation activates the UPR, suggesting a role of H6PD in ER homeostasis. Here, we evaluated whether hnRNPK regulates UPR through H6PD.
Materials and Methods: We performed RNA immunoprecipitation (RIP) to validate the interaction of hnRNPK with H6PD mRNA in HCT116 cells. hnRNPK overexpression or downregulation was carried out in CRC cells to evaluate H6PD mRNA levels and UPR markers by qPCR and western blot, respectively. Thapsigargin was used to induce ER stress and to evaluate hnRNPK and H6PD levels in CRC cells.
Results: The RIP analysis confirmed the interaction between hnRNPK and H6PD. Additionally, we found that hnRNPK increases H6PD mRNA levels in CRC cells. On the other hand, hnRNPK downregulation increased the expression of UPR markers. To validate the association of hnRNPK with the UPR response, we evaluated the mRNA and protein levels of hnRNPK and H6PD under ER stress, confirming they increase.
Discussion: Our results indicated that hnRNPK is involved in the UPR response by regulating H6PD expression, suggesting that hnRNPK maintains ER homeostasis in CRC cells.
Acknowledgment: Fondecyt 1201215, 1241771

9. Role of the Carrot DcPIF3 in Regulating Carotenoid Biosynthesis During Photomorphogenesis in Plants. Arancibia-Aguilera, Nicolás; Mora, Patricio; Arias,

Daniela; González-Calquín, Christian, and Stange, Claudia. Plant Molecular Biology Centre, Department of Biology, Faculty of Sciences, Universidad de Chile, Las Palmeras 3425, Ñuñoa, Santiago, Chile

Introduction: During photomorphogenesis in plants, light plays a crucial role in regulating gene expression and biosynthesis of chlorophylls and carotenoids. PIFs (Phytochrome Interacting Factors) are bZIP transcription factors described as negative regulators of photomorphogenesis and carotenoid synthesis in plants. In *Arabidopsis thaliana*, AtPIF1 is active in the dark but inactivated by photoreceptors such as AtPHYA and the co-factor PAR1 when plants are in shade, which allows the expression of carotenoid biosynthetic genes such as *AtPSY*. *Daucus carota* (carrot) storage roots accumulates high levels of carotenoids when it grows underground and has been demonstrated that DcPHYA and DCPAR1 are required for this process. By means of RNA-seq analysis, we determined that *DcPIF3* is highly expressed in the taproot grown underground.
Methods: By predicting functionality through bioinformatics, subcellular localization in tobacco leaves, DcPIF3 overexpression in *A. thaliana*, an *in vivo* expression assay and ChIP-PCR we aimed to determine the *in vivo* functionality of DcPIF3.
Results: *In silico* analysis revealed that DcPIF3 has 48% identity with AtPIF3, retaining DNA and protein binding domains. Fluorescence microscopy showed that DcPIF3 localizes in the nucleus, interacting as homodimers and heterodimers with DcPAR1. Relative expression of *DcPIF3* was higher in carrot roots grown in darkness, aligning with previous transcriptomic data. Transgenic *Arabidopsis* plants overexpressing 35sCaMV:DcPIF3 exhibited significant stem elongation compared to control plants, reduced *AtPSY* transcript levels, and decreased total carotenoid accumulation. ChIP assays demonstrated specific binding of DcPIF3 to G-boxes in the *AtPSY* promoter in dark-grown plants, indicating its repressive role in carotenoid biosynthesis in *A. thaliana*. In *Nicotiana tabacum*, transient overexpression of 35S:DcPIF3 via agroinfiltration significantly repressed *DcPSY1, DcPSY2* and *NtPSYs* gene expression.
Discussion: These findings suggest that DcPIF3 is a conserved negative regulator of carotenoid biosynthesis, emphasizing its role in photosynthetic adaptation.
Funding: Fondecyt 1221399

10.Pyruvate kinase from methanogenic archaea: AMP activation and structural insights through X-ray crystallography. Ignacio Aravena-Valenzuela (ignacio.aravena.v@ug.uchile.cl), Sebastián M. Muñoz, Felipe Gonzalez-Ordenes, Antonia Alarcón-Saavedra, Gabriel Vallejos-Baccelliere, Víctor Castro-Fernández, Victoria Guixé. Laboratorio de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Chile.

Central carbon metabolism pathways in Archaea, such as glycolysis, differ from those in Bacteria and Eukaryotes, including their allosteric effectors. The final step of glycolysis is catalyzed by pyruvate kinase (PK), 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, the PK enzyme from the hyperthermophile *Methanocaldococcus jannaschii* (MjPK) is described as activated by AMP. In contrast, the enzyme from the anaerobic organism *Methanococcus maripaludis* (MmPK) has been reported as not activated. To address these differences from a structural and kinetic point of view, the MjPK and MmPK enzymes were recombinantly expressed, purified, and characterized. Multiple-angle light scattering and circular dichroism spectra revealed that both enzymes are folded tetramers in solution. Kinetic characterization shows that MjPK and MmPK display cooperative kinetics for the substrate phosphoenolpyruvate. AMP activates both enzymes by reducing the half-saturation constant for phosphoenolpyruvate and concomitantly decreasing the Hill number, being this effect more pronounced in the MjPK enzyme. Additionally, we determined the structure of both enzymes by X-ray crystallography, revealing the presence of a disulfide bond in the lid domain from MmPK, which closes over the active site after substrate binding. Considering the anaerobic physiological context under which MmPK operates, these cysteines should be reduced. Consistent with this, reducing conditions, such as the addition of a reducing agent, increase the activating effect of AMP in MmPK. Our findings underscore the relevance of AMP as a major regulator of glycolytic metabolism in methanogenic archaea, as it has also been found to activate the glucokinase and phosphofructokinase activities of these organisms. This study contributes to the understanding of methanogenic archaea's central carbon metabolism, which currently lacks structural and regulatory information.
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11. Next-generation agriculture bioactives: Lipid nanoparticles containing miRNAs to biofortify crops using *Medicago truncatula* and its symbiotic interactions as model holobiont. Karla Araya-Castro (karla.araya@ufrontera.cl)[1,2], Rowan Herridge[3], Benjamín Durán-Vinet[4], Tzu-Chiao Chao[5], Lynette Brownfield[3], Richard C. Macknight[3], Arlene McDowell[6], María de la Luz Mora[1,2]. [1]Scientific and Technological Bioresource Nucleus (BIOREN-UFRO), Universidad de La Frontera, Temuco 4780000, Chile. [2]Centre of Plant, Soil Interaction and Natural Resources Biotechnology, Universidad de La Frontera, 4780000, Chile. [3]Department of Biochemistry, University of Otago, Dunedin 9054, New Zealand. [4]Department of Anatomy, School of Biomedical Sciences, University of Otago, Dunedin 9054, New Zealand. [5]Institute of Environmental Change & Society, Department of Biology, University of Regina, Regina, SK S4S, Canada. [6]School of Pharmacy, University of Otago, Dunedin 9054, New Zealand.

Introduction: Lipid nanoparticles (LNPs) have shown significant promise in medical drug delivery, yet their application in agriculture for systematic bioactive delivery in plants remains largely unexplored. LNPs can efficiently deliver their cargo intracellularly, presenting an opportunity to enhance crop traits such as nutrient content, growth, yield, and fruit quality. Additionally, the role of microRNAs (miRNAs) in regulating plant interactions with arbuscular

mycorrhizal fungi (AMF) and rhizobia (RZ) is an emerging and under-researched field. Recent studies highlight miRNAs as key regulators of these interactions through mRNA cleavage. This work aims to evaluate the effectiveness of LNPs in delivering miRNAs that modulate plant interactions with AMF and RZ, potentially advancing crop biofortification and productivity.
Materials and Methods: *Medicago truncatula* miRNA (miR2111) was designed and screened using Geneious Prime and miRbase. Final miRNAs were synthesized as duplexes with 2 nucleotide overhangs in the 5´and 3´ends. These miRNAs were entrapped in LNPs, which were synthesized using Nanoassemblr and characterized using dynamic light scattering (DLS), and transmission electron microscopy (TEM). The formulations were then tested through drip irrigation during seed germination and foliar applications to evaluate their impact on lateral root formation, and nodulation.
Results: Obtained LNPs had an entrapment efficiency (EE) of 70.5%, with a diameter of 122.5 ± 0.9 nm, and a Polydispersity Index (PDI) of 0.1. A significant increase of nodules and lateral roots was observed in *M. truncatula* plants sprayed with the formulation of LNPs loaded with miRNA compared to control plants.
Discussion: Results demonstrate that this approach could revolutionize crop biofortification, with potential applications to other crops such as wheat, oat, and maize. The novel delivery system could offer a significant advancement in agriculture, addressing challenges related to nutrient deficiencies, drought, and diseases, and enhancing crop resilience and productivity in the context of climate change.
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12. HY5, a key transcription factor in light signaling pathway: a study of its response to salt stress in Microtom tomato plants. Daniela Arias-G[1] (danielaloreto.arias@gmail.com), Sebastián Velozo[2], Nicolás Arancibia-A[1] and Michael Handford[1]. [1]Centro de Biología Molecular Vegetal (CBMV), Universidad de Chile. [2]Centro de Biotecnología Vegetal (CBV), Universidad Andrés Bello.

Light is crucial for photosynthesis and photomorphogenesis in plants. The transcription factor (TF) HY5 (ELONGATED HYPOCOTYL 5) is a key positive modulator of photomorphogenesis, regulating the transcription of light-responsive genes by binding to their promoters. Salt stress induces Abscisic Acid (ABA) production, activating a signaling pathway composed of ABA-responsive genes that help cope with such stress. HY5 binds to promoters of genes involved in ABA biosynthesis and signaling, suggesting a role in salt stress responses. However, little is known about the relationship between HY5 and salt stress in adult plants. This study characterized the HY5 response in tomato subjected to salt stress. *HY5* transcript levels increase in plants treated with 100 or 200 mM NaCl (48h post-treatment, 16h light/8h dark photoperiod), correlating with increased transcripts of *ABI5* and

NCED, genes involved in ABA signaling and biosynthesis, respectively. HY5 protein levels also increase in NaCl-treated plants compared to controls without salt, peaking at 24h post-treatment, indicating a lack of correlation between HY5 transcript and protein levels. As *ABI5* and *NCED* promoters contain light-responsive *cis*-elements to which HY5 could bind, the ability of this TF to bind these sequences is being evaluated. Additionally, the ratios of red (R) to far-red (FR) light alter *HY5* expression and abundance profiles in response to NaCl. A low R:FR ratio promotes HY5 accumulation under salt stress, with a peak at 48h post-treatment (without changes in transcript levels), in contrast to plants grown under normal photoperiod conditions. In higher R:FR ratios, HY5 abundance decreases. These results suggest that HY5 is involved in salt stress responses, possibly by activating the ABA signaling pathway. The expression and accumulation profile of HY5 is modified according to the R:FR ratio, suggesting that light may influence the salt stress response mediated by HY5 in plants.
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13.Transcriptional regulation of STAT3 in immune response pathways associated to modulation of HDAC6 in colorectal cancer cell lines. M. Estrella Armijo (marmijo@ucsc.cl), Constanza Mardones, Yanitza Gutiérrez, Carla Maldonado, Carlos Vivar, Michel Pradenas y Matías I. Hepp. Laboratorio de Investigación en Ciencias Biomédica, Departamento de Ciencias Básicas y Morfología, Facultad de Medicina, Universidad Católica de la Santísima Concepción, Concepción, Chile.
Introduction: Colorectal cancer (CRC) has one of the highest incidental rates among other types of cancer worldwide. Despite having several treatments available against CRC, many patients do not respond well to the therapy or experience considerable side effects. However, using immunotherapy to target the immune-checkpoints modulators deregulated in CRC, might be an alternative treatment that improves the patients' outcomes. Interestingly, 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, as well as the expression of genes involved in immune response. Nevertheless, the effects of HDAC6 in the STAT3 pathway in CRC is not well-known. For this purpose, we analyzed the transcriptional targets of the HDAC6/STAT3 axis, associated with immune response, in CRC cell lines.
Materials and Methods: We treated the cell with a selective HDAC6 inhibitor (Nexturastat A), Interleukin-6 to emulate a pro-inflammatory environment, and a STAT3 inhibitor (Istat3). Then, we evaluated the expression levels of immunomodulators and immune response genes by RT-qPCR. Furthermore, we examined by Western blot the protein levels of PD-L1, a known transcriptional target of STAT3, as well as the HDAC6/STAT3 protein interaction by Co-immunoprecipitation assays.
Results: The results suggest that some immune response pathways are affected in CRC due to the increase in the expression levels of immunomodulator genes when cells are treated with Interleukin-6. Whereas STAT3 is inhibited by Istat3 (directly) or Nexturastat A

(indirectly), affecting the interaction of HDAC6 with STAT3 and decreasing the expression levels of immunomodulatory genes, which can improve the immune response against CRC cells.
Discussion: Therefore, it is suggested that the inhibition of HDAC6 could be used for immunotherapy in CCR as a novel co-adjuvant therapeutic strategy.
Funding: UCSC (FAA2024); FONDECYT de Postdoctorado 2024 (3240256).

14. An approach to use the Energy Dispersive micro X-Ray Fluorescence spectrometry to determine element variation and distribution in plant response to salt stress. Oscar Arrey-Salas[1], Yasnaya Bolua[1,2], Mónica Yánez[1], Roberto Miño[2], Ricardo Cabeza[3], Alex San Martín[1], Simón Ruiz[4], Erwan Michard[1]. [1]Laboratory of Plant Cell Sensing and Signalling (PCSS Lab), Instituto de Ciencias Biológicas, Universidad de Talca, Talca 3460000, Chile. [2]Doctorado en Ciencias mención Biología Vegetal y Biotecnología, Universidad de Talca, Talca 3460000, Chile. [3]Faculty of Agricultural Sciences, University of Talca, Talca, Chile. [4]Laboratorio de Genómica Funcional, Instituto de Ciencias Biológicas, Universidad de Talca, Talca 3460000, Chile

Saline stress affects ion homeostasis in plants by causing excessive accumulation of sodium (Na^+) and chloride (Cl^-) ions, which compete with essential nutrients such as potassium (K^+) and calcium (Ca^{2+}), disrupting their absorption and affecting biochemical processes. The elemental analysis of plant material is crucial for understanding these effects, but methods for the quantification of the ionome, or the elemental composition of an organism, are currently limited to costly methods that require large amounts of plant tissue. In this context, the Energy Dispersive micro X-Ray Fluorescence (μ-EDXRF) technique has proved particularly effective in the study of elemental distribution in different organs of plants exposed to different elemental conditions. This technique provides 2D maps of the elemental spatial distributions and quantification in terms of intensity or photon count but does not provide concentration units unless it is combined with Inductively Coupled Plasma Mass Spectrometry (ICP-MS).
This work aims to develop a methodology for detecting the effect of salt stress on the plant ionome using a Fundamental Parameter (Standardless) based analysis with a commercial benchtop Bruker M4 Tornado μ-EDXRF spectrometer. First, the limit of detection (LOD) of the equipment was determined using increasing concentrations (from 0 to 3-5M) of different salts on a filter paper matrix, and the counts of photons per seconds (cps/keV) values were correlated with the known concentrations for linear regression of the data. To measure the element concentration in response to salt stress, the spectrometer uses its own standardless semi-quantitative M4 Tornado software (based on ESPRIT) package with reliability and reproducibility.
we test this methodology by subjecting Arabidopsis, tomato, and the mosses *Physcomitrium patens* and *Sanionia uncinata* to a saline condition. Finally, we compared the differential response between Col-0 and Arabidopsis plants overexpressing vesicular trafficking genes.

Here, we present imaging of the elemental distributions of key elements and the calculated concentrations of Na, Cl, K, P, and Ca. In aerial tissue, treatments caused a significant increase in Na and Cl across all conditions, while the concentration of K decreased significantly. The pattern of Ca accumulation was unclear across all treatments, warranting further investigation. Overexpression of individual genes involved in vesicular trafficking significantly altered ion accumulation in Arabidopsis.
We show that using an optimized benchtop machine with protocols for measurement and quantification tailored for plant analyses, ionome distribution can be investigated in a reliable, efficient, and cost-effective manner. This has high potential to contribute to a better understanding of plant physiology and to improve breeding programs and agriculture.
Funding: This work was supported by Fondecyt Regular1210920.

15. Unveiling Microbial Diversity: Cost-Effective 16S rRNA Amplicon Sequencing using DeCodi-Fi High Fidelity Polymerase. Isidora Arriagada (i.arriagada@kurabiotech.com) J. Gimpel, J. Hsieh, J. Sáez, J. Cáceres, I. Arriagada, S. Kamkar, I. Marx, M. Rozas. Kura Biotech Spa, Puerto Varas, Región de Los Lagos, Chile

Introduction: 16S rRNA amplicon sequencing is essential for surveying microbial communities. The choice of PCR amplification conditions can significantly impact microbial representation. This study compares the performance of 8 different polymerase/buffer combinations using 2 mock communities and 2 real feces samples to examine their effect on relative abundance profiles, number of species detected and chimera formation. Consequently, it is valuable to contrast these technical results with the cost-effectiveness of each polymerase.
Materials and Methods: Templates included two mock community standards and two real stool samples for 16S V4 and V3-V4 region amplification. Eight different polymerase/buffer combinations were tested following each provider instructions. PCR product was purified, quantified, and sequenced. Data analysis was performed using the DADA2 workflow and SILVA 16S database.
Results: DeCodi-Fi polymerase, an affordable high-fidelity option, showed performance comparable to more expensive DNA polymerase enzymes (HiFi K, HiFi S, HiFi Q, Taq T, and Taq N). Except for Taq N, all treatments provided similar 16S rRNA sequencing data. DeCodi-Fi and HiFi K had lower chimera rates compared to HiFi S and Q. PCR cycle number (25 vs 30) did not significantly impact relative abundance.
Discussion: These findings underscore the importance of careful polymerase selection in 16S microbiome studies, highlighting the economic viability and performance gain of using DeCodi-Fi polymerase in this context.

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16. The ITAF function of hnRNPA1 and HuR over the HIV-1 IRES depends on the

cellular context. Patricio Astudillo (pdastudi@uc.cl), Marcelo López-Lastra. Laboratorio de Virología Molecular, Instituto Milenio de Inmunología e Inmunoterapia, Departamento de Enfermedades Infecciosas e Inmunología Pediátrica, Escuela de Medicina, Pontificia, Universidad Católica de Chile.

Introduction: The human immunodeficiency virus type 1 (HIV-1) full-length mRNA (vRNA) can initiate translation using an internal ribosome entry site (IRES). How the HIV-1 IRES recruits the host translation machinery remains poorly understood. Nonetheless, HIV-1 IRES requires cellular proteins, IRES transacting factors (ITAFs), to be functional. In this study, we use natural variants HIV-1 VAR 2 IRES and HIV-1 VAR 14 IRES, isolated from vRNA recovered from infected patients, to further understand how cellular proteins modulate HIV-1 IRES activity in different cellular contexts. HIV-1 VAR 2 IRES has an increased (400%), while HIV-1 VAR 14 IRES has a decreased (17%) activity when compared to the prototypic HIV-1 IRES isolated from pNL4.3 (100%). The heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1), a stimulatory ITAF, and human antigen R (HuR), an inhibitory ITAF, were selected as proteins.
Materials and Methods: Vectors expressing hnRNPA1 or HuR proteins were co-transfected in cells together with the bicistronic vector dl HIV-1 VAR2 and dl HIV-1 VAR14 IRES or dl HIV-1IRES, harboring the HIV-1 5'UTR, VAR2, VAR14 or pNL4.3 vRNA in their intercistronic region, respectively. Western blot assays confirmed the expression of hnRNPA1 and HuR, and luciferase activities were determined.
Results: hnRNPA1 differentially enhanced HIV-1 VAR2 IRES, HIV-1 VAR 14 IRES, and HIV-1 IRES translation initiation in HEK293T and HeLa cells. In contrast, HuR reduced HIV-1 IRES-dependent translation initiation independent of the cellular context.
Discussion: Based on these findings, we conclude that hnRNPA1, but not HuR, regulates HIV-1 IRES-mediated translation initiation in a cell type-specific manner. Furthermore, data highlight that the natural sequence variability of HIV-1 impacts HIV-1 IRES activity and its modulation by cellular proteins.
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17. Evaluation of a combined approach of techniques for the detection and diagnosis of *H. pylori* in gastric cancer tissue. Cecilia Barrera-Barrios[1,2] (cecilia.barrera@falp.org) [&], Sebastián Estay-Serey[1,2] (sebastian.estay@falp.org) [&], Joaquín Reyes-González[2], and Franz Villarroel-Espindola[2]. [1]San Sebastián University, Faculty of Medicine and Science, Medical Technology School. [2]Traslational Medicine Unit, Fundación Arturo López Pérez (FALP), Santiago, Chile.

Introduction: The best-known risk factor of gastric cancer (GC) is *Helicobacter pylori* (*HP*). The reported prevalence of *HP* infection in Chile reaches 75%. Endoscopy, rapid urease

test, histology, microbiological culture and PCR have been implemented for the diagnosis of *HP*-infection in non-oncological patients. Very few reports have included two or more methodologies and oncological cases. The aim of this study was to evaluate the value of combining histological and molecular biology tools to detect *HP* in gastric tumors.
Materials and Methods: 53 formalin-fixed and paraffin-embedded (FFPE) surgical resections were studied using immunofluorescence against *HP*-antigens and PCR to detect *HP-UreC* gene, and compared to Giemsa staining as gold standard. The validity and clinical value of different methodologies were evaluated by correlation analyses.
Results: The correlation between the studied techniques showed a Cohen's Kappa index for Giemsa/PCR of 0.18 (weak agreement); for Giemsa/IF of 0.49 (moderate agreement) and for PCR/IF of 0.2 (weak agreement). The degree of sensitivity and specificity between Giemsa/PCR were 88% and 30%; for Giemsa/IF 92% and for PCR/IF 53% and 84% and 39%, respectively. Finally, the positive and negative predictive values for Giemsa/PCR were 54% and 72%; for Giemsa/IF 83% and 73%, and for PCR/IF 55% and 73%, respectively.
Discussion: Our PCR requires further studies to improve its reliability. Giemsa and immunofluorescence showed to be the most optimal combination. The combined use of histological staining and molecular biology techniques increases the sensitivity for the diagnosis of *HP* infection in GC FFPE samples.
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18. Cardioprotective effect of VCAM-1 on norepinephrine-induced hypertrophic female cardiomyocytes. Elsa Rocío Bascuñán[1] (elsa.bascunan@ug.uchile.cl), Ximena Calle-Chalco[1], Erik López[1], Mayarling F Troncoso[1], Laura Navarrete-Gallegos[1], Ingrid Oyarzún[2], Magda C. Díaz-Vesga[1], Fernanda Zapata-Neweu[1], Brenda Becerra-Leiva[1], Danica Jimenez-Gallegos[1], Francisco Pino de la Fuente[1], Angélica Ortega-Muñoz[1], Claudia Muñoz[13], Alejandra Guerrero-Moncayo[1], Clara Quiroga[2], Mario Chiong[1], Sergio Lavandero[1,3]. [1]Advanced Center of Chronic Diseases (ACCDiS), Facultad de Ciencias Químicas y Farmacéuticas & Facultad Medicina, Universidad de Chile, Chile. [2]Advanced Center of Chronic Diseases (ACCDiS), Facultad Medicina, P. Universidad Católica de Chile, Chile. [3]Cardiology Division, University of Texas Southwestern Medical Center, Dallas, USA

Introduction: Hypertension-induced organ damage, particularly in the heart, occurs through various mechanisms, including increased catecholamine levels (norepinephrine and epinephrine). Additionally, there are gender-related differences mainly attributed to circulating estradiol. This steroid hormone has a cardioprotective effect during a woman's fertile years. In postmenopausal women, morphological changes occur in the heart, such as left ventricular hypertrophy. Moreover, postmenopausal women with hypertension have high plasma concentrations of vascular cell adhesion molecule 1 (VCAM-1), suggesting a potential link to cardiovascular risk. We previously reported that cardiac VCAM-1 also has a

cardioprotective action in the heart. However, it remains unclear if the cardioprotective effect of estrogen on the heart could be mediated by cardiac VCAM-1.
Methods: Neonatal ventricular cardiomyocytes (NRVM) were obtained from male and female 1-2 day old Sprague-Dawley rats (Bioethics protocol 21518-CQF-UCH). Cells were prepared using collagenase digestion and cultured in Dulbecco's Modified Eagle's Medium (DMEM). Cardiomyocytes were treated with 10 µM norepinephrine (NE) for 24 h to induce hypertrophy. Cardiomyocytes were then processed for protein extraction using the RIPA protocol, and atrial natriuretic peptide (ANP) and VCAM-1 were detected by western blotting.
Results: Differences between male and female cardiomyocytes under NE treatment were observed. We found that male cardiomyocytes increased the hypertrophic marker ANP, but this change was not observed in female cardiomyocytes. Additionally, the protein levels of VCAM-1 in female cardiomyocytes were elevated compared to male cardiomyocytes, treated and not treated with NE.
Discussion: The data suggest that NE induces cardiomyocyte hypertrophy in a gender-specific manner. This difference was also observed in VCAM-1. Further experiments are required to assess whether VCAM-1 is responsible for the NE-dependent sex-specific hypertrophic response. Molecular mechanisms underlying this response will be studied.
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19. Role of Transcription Factor SALL2 in Suppressing Epithelial-Mesenchymal Transition and Malignancy in Colorectal Cancer. Diego Benítez-Riquelme[1](diegobenitez@udec.cl), Manuel Mastel[2], Aracelly Quiroz[3], David Peña[1], Carolina Delgado[3], Rene Jackstadt[2], Ariel Castro[1], Iván Gonzalez-Chavarría[4] & Roxana Pincheira[1]. [1]Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Chile. [2]Cancer Progression and Metastasis Group, German Cancer Research Center (DKFZ), Heidelberg, Germany. [3]Sección Patología, Departamento de Especialidades, Facultad de Medicina, Universidad de Concepción, Chile. [4]Departamento de Fisiopatología, Facultad de Ciencias Biológicas, Universidad de Concepción, Chile.

Introduction: EMT is a fundamental process in cancer progression, triggering metastasis by enabling epithelial cells to acquire mesenchymal properties. SALL2 is a transcription factor that regulates cell proliferation, death, and migration. Our previous studies showed that SALL2 decreases progressively from normal colon tissue to adenocarcinoma, suggesting a tumor suppressor role in colorectal cancer (CRC). However, there are no functional studies of SALL2 in CRC. This study aims to elucidate the role of SALL2 in regulating EMT and its impact on CRC malignancy using different approaches. **Materials and Methods**: We used loss- and gain-of-function of SALL2 in SW480 and SW620 cells. Assays included proliferation, transwell migration, colony formation, and EMT marker evaluation via western blot and qPCR. Immunocytochemistry assessed vinculin and F-actin fluorescence, and nuclear morphology. In 3D models, we generated *Sall2* knockout in AKP

mouse organoids (altered *Apc*, *KRas*, and *Trp53*). We evaluated EMT markers by qPCR, growth and viability. *In vivo* studies involved colonoscopy-guided microinjections of these organoids, assessing tumor growth, survival, and H&E staining for tissue invasion.
Results: SALL2 loss of function increased proliferation, migration, and EMT markers (e.g., Snail, N-cadherin, and vinculin), and was associated with elongated and smaller nuclei. *Sall2* loss increased growth, viability, and EMT markers of organoid models. *In vivo*, *Sall2*-deficient tumors grew faster and reduced survival rates. H&E staining from *Sall2*-deficient tumors showed deeper invasion into muscular layers and lamina propria.
Discussion: Results indicate that SALL2 suppresses EMT and malignancy in CRC. SALL2 deficiency enhances proliferation, migration, EMT marker expression, and organoid viability. *In vivo*, loss of SALL2 leads to aggressive tumor growth, reduced survival, and deeper invasion. These findings suggest SALL2 as a critical regulator of cellular plasticity and a potential biomarker and therapeutic target. Future research should investigate molecular mechanisms of SALL2 in EMT regulation and its role in other cancers.

20. Kinetic characterization of the thermo-alkaline recombinant lipase Lip7. Hardy Guzmán[1], Diego Salas-Bruggink[2], Jenny M. Blamey[1,2] (jenny.blamey@usach.cl). [1]Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile. [2]Fundación Científica y Cultural Biociencia, Santiago, Chile.

Introduction: Lipases (EC 3.1.1.3) are lipids degrading enzymes commonly used in different industrial processes, as in food and pharmaceutical industries. Lipases have preference to long chain substrate, however, promiscuous activity hydrolyzing short-chain lipids has been reported. Promiscuous enzymatic activity is not rare distributed in a wide range of organisms. Extremophiles has become an important source of new biocatalyst since the wide range of activity in different conditions such as pH and temperature, which makes it versatile and valuable for industrial applications. Thus, the aim of this study is to characterize the substrate specificity of the lipase from extremophile microorganism.
Materials and methods: We characterized the thermo-alkaline recombinant lipase Lip7, obtained from *Geobacillus sp.* ID-17, using p-nitrophenyl (pNP) derived esters of model fatty acids (pNP acetate, pNP butyrate, pNP valerate, pNP octanoate, pNP laurate, pNP myristate and pNP palmitate).
Results: The saturation curves were adjusted to allosteric sigmoidal model, which is consistent with the interfacial activation. The interfacial activation is a unique characteristic of lipases, which increase the activity of lipases at aqueous-lipid interfaces. Lip7 exhibits a preference for medium chain substrates, specifically pNP laurate, which has the highest kinetic efficiency (k_{cat} /$K_{1/2}$=208 $s^{-1}M^{-1}$).
Conclusions: Lip7 kinetic characterization and substrate specificity could improve our understanding of its potential industrial applications.
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21. Arabidopsis OGOX1 Enhances Susceptibility Against *Myzus persicae* Through Oligogalacturonides Oxidation Released During Plant-Aphid Interaction. Francisca Blanco-Herrera[1,2,3,6] (mblanco@unab.cl), Diego Zavala[1,2,3], Carla Zúñiga-Pablopulos[1], Fernando José Bustos[4,5]. [1]Universidad Andrés Bello, Centro de Biotecnología Vegetal, Facultad de Ciencias de la Vida, Santiago 8370186, Chile. [2]Millennium Science Initiative Program (ANID), Millennium Institute for Integrative Biology (iBio), Santiago, Chile. [3]Millennium Science Initiative Program (ANID), Millennium Nucleus for the Development of Super Adaptable Plants (MN-SAP), Santiago, Chile. [4] Universidad Andrés Bello, Instituto de Ciencias Biomédicas, Facultad de Medicina y Facultad de Ciencias de la Vida, Santiago 8370071, Chile. [5]Millennium Nucleus of Neuroepigenetics and Plasticity (EpiNeuro), Santiago, Chile. [6]Center of Applied Ecology and Sustainability (CAPES), Pontificia Universidad Católica de Chile, Santiago, Chile.

Aphids, such as *Myzus persicae*, are important, globally distributed agricultural pests that cause significant economic losses worldwide by feeding on plant phloem. Aphids have evolved mechanisms to access internal tissues and feed on the host plant, beginning when their mouthparts penetrate the cuticle and then move into the phloem through intercellular pathways such as cell wall matrices until reaching the vascular tissue. The ability of host plants to detect aphid feeding and mount an effective and rapid defense is a critical factor in determining their resistance or susceptibility. Here we investigate how pectin dynamics affect plant host resistance to aphids. Pectin is one of the most abundant polysaccharides on secondary cell walls, consisting mainly of homogalacturonan (HG) domains. Its fragmentation during pathogenesis leads to the release of oligogalacturonides (OGs), which can trigger several defense responses in plants and increase resistance against aphids. We measured the activity of HG-remodeling enzymes in Arabidopsis plants infested with *M. persicae* to evaluate OGs release. We detect increased total pectin methylesterase enzymatic activity, indicating HG demethylesterification during infestation and subsequent hydrolysis by pectate lyase enzymes. In addition, we detected an increase in transcript levels of an oligogalacturonide oxidase, suggesting oxidation of OGs during aphid infestation. In Arabidopsis, OGOX1 controls the inactivation of OGs-induced defense responses by oxidizing their reducing ends. To evaluate whether OGs oxidation affects aphid performance, we generated *ogox1* knockout plants using CRISPR-Cas9 and measured the number of offspring when aphids were forced to feed on these plants. In addition, we used electrical penetration graph analysis to describe aphid feeding behavior in *ogox1* mutant plants. This work aims to determine how plants sense aphid infestation by recognizing OGs and controlling defensive responses through the catalytic activity of OGOX1.

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22. Molecular and cellular functions of *Physcomitrium patens* GLR ion channels in stress sensing and response. Yasnana Bolua Hernández[1,2] (yasnayabolua@gmail.com), Fernando Vergara-Valladares[3], Ingo Dreyer[3] and Erwan Michard[2]. [1]Doctorado en Ciencias mención Biología Vegetal y Biotecnología, Universidad de Talca, Talca 3460000, Chile. [2]Laboratory of Plant Cell Sensing and Signalling (PSS Lab), Instituto de Ciencias Biológicas, Universidad de Talca, Talca 3460000, Chile. [3]Civil Engineering Faculty, Universidad de Talca, Talca 3460000, Chile.

Plant glutamate receptor-like channels (GLR) are homologous to animal ionotropic glutamate receptors (iGluR). The latter play a key role in almost all aspects of brain functions, including memory and learning. In higher plants, GLRs participate in many functions including pollen tube growth, root growth, development and regeneration, stomatal opening, and response to pathogens. In moss, GLRs have been involved in sperm cell chemotaxis and abiotic stress response. Recently, GLRs have attracted attention for their role in long-distance electric and second messenger calcium (Ca^{2+}) signaling in response to biotic or abiotic stresses. This Ca^{2+} signal acts synergistically with a second signal, characterized by a change in redox potential, involving the synthesis of H_2O_2 by membrane proteins (RBOH) and the synthesis of molecules with high reducing power (NADPH, GSH and Ascorbic Acid). Preliminary data in heterologous system suggest a regulation of GLR by redox potential, and particularly H_2O_2 and GSH. Here, we look for a molecular mechanism of GLR regulation by redox potential. We generated GLR mutants for extracellular cysteines potentially involved in channel regulation and expressed those mutants in HEK293 mammalian cells. GLR expressing cells were subjected to both Ca^{2+} imaging and patch-clamp analysis in the whole cell configuration. We observed a differential regulation by redox potential of the mutant channels versus wild types and propose a molecular model for this regulation. Taken together, our experiments contribute to the understanding of the regulation of GLR channels, that play key role in plant cell stress sensing and response. We conclude with a discuss the physiological implication.
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23. Adjuvant Effect of Maraviroc in Combination with Cisplatin on Tumor Growth and Survival in a Murine Model of Gastric Cancer. Brebi Priscilla[1, 2, 3], Mora Barbara[4], Reyes María[4], Ili Carmen[1, 2, 3], Mora Yuselin[1], Tatiana Mellipan[1], Buchegger Kurt[2,3,5]. [1]Laboratory of Integrative Biology (LIBi), Center of Excellence in Translational Medicine (CEMT), Scientific and Technological Bioresource Nucleus (BIOREN), Universidad de La Frontera, Temuco 4780000, Chile. [2]Millennium Institute on Immunology and Immunotherapy, Santiago, Chile. [3]BMRC, Biomedical Research Consortium-Chile. [4]Institute of Biomedical Sciences, Faculty of Health Sciences, Universidad Autónoma de Chile, Temuco, Chile. [5]Department of Basic Sciences, School of Medicine, Universidad de La Frontera, Temuco, Chile.

Introduction: Chemoresistance remains a significant challenge in the treatment of gastric cancer, leading to poor prognosis and limited therapeutic options. The CCL5/CCR5 axis has been implicated in tumor progression and resistance to chemotherapeutics. Given the critical role of this axis in cancer progression, this study evaluated the potential of Maraviroc (MVC), a CCR5 antagonist, as an adjuvant to cisplatin (CDDP) to overcome chemoresistance in a murine model of gastric cancer.
Materials and Methods: A murine xenograft model using gastric tumor cells was employed. Mice were treated with MVC, CDDP, or a combination of both. Tumor growth and survival were monitored for 21 days. Biochemical analyses were performed to assess the metabolic profile of the animals.
Results: The combination of MVC and CDDP did not induce a greater reduction in tumor volume compared to CDDP alone. However, the co-administration of MVC significantly improved the survival of treated animals. Additionally, the combination attenuated the adverse effects of CDDP on biochemical parameters such as glucose, lactate, and creatinine levels.
Discussion: The results suggest that MVC could act as a sensitizing agent, enhancing the efficacy of CDDP while reducing its toxicity. Although the exact mechanism of this synergy is not yet fully elucidated, it is hypothesized that blocking CCR5 could modulate the tumor immune response and reduce resistance to chemotherapy. These findings open new avenues for the development of combined therapeutic strategies for the treatment of gastric cancer. Future studies should delve deeper into the underlying molecular mechanisms and evaluate the efficacy of this combination in more complex preclinical models.
Acknowledgment: Projects: FONDECYT 1210440, CORFO 23CTEC-250091 and Millennium Institute on Immunology and Immunotherapy (IMII) (No. ICN2021_045).

24. Endothelin-converting enzyme isoforms in cancer models. David Brown-Brown[1](david.andres.brown@gmail.com), Jetzabel Vidal-Vidal[1], Carlos Spichiger[1], Julio Tapia [2], Ignacio Niechi[1]. [1]Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile. [2]Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Santiago, Chile.

Introduction: Endothelin-converting enzyme 1 (ECE1) is a crucial enzyme involved in tumor progression. ECE1 exists as four isoforms (a-d) generated through alternative splicing and differing only in their cytoplasmic N-terminal domains. Elevated levels of total ECE1 transcript and protein have been observed in various cancer types; however, there is a lack of specific antibodies for isoform detection, and no established quantitative method exists for comparing the expression of transcript variants. While all isoforms share the same catalytic domain, it has been proposed that they may have unique isoform-specific roles beyond this activity. Therefore, understanding the expression patterns of each isoform is of significant interest.

Methods: This study aims to develop and standardize a quantitative qPCR method for measuring ECE1 isoforms expression in lung, colon and gallbladder cancer cells. Protein levels were measured by western blot.
Results: We have developed a quantitative RT-qPCR method utilizing the $2^{-\Delta ct}$ approach to profile the expression of ECE1 transcript variants. Protein levels of ECE1 were measured by western blot using a pan-ECE1 antibody. Our results shown that ECE1c is the most prevalent isoform in tumor models, while ECE1a is the least abundant across most cell types.
Discussion: This method enables the quantitative determination of ECE1 variants, highlighting a potential predominant role of the ECE1c isoform. This approach facilitates the molecular investigation of ECE1 role and its association with tumor progression.
Acknowledgment: ANID-FONDECYT 11220149 (IN).

25. Identification of MHC-II-restricted immunogenic peptides from mollusk hemocyanins using bioinformatic tools and *in vitro* validation in antigen-presenting cells. Javier Bustamante C.[1], Michelle L. Salazar[1], Augusto Manubens[1,2], Fabián Tempio[3], Felipe Vergara[1], Claudia d'Alencon[1], Fermín E. González[3], Flavio Salazar-Onfray[4], Iván Flores[4], Diego Díaz-Dinamarca[1], Fabián Salazar[1,5], María Inés Becker[1,2]. [1]Laboratory of Immunology, Fundación Ciencia y Tecnología para el Desarrollo (FUCITED). [2]Laboratory of Research and Development, Biosonda S.A. [3]Laboratory of Experimental Immunology & Cancer, Faculty of Dentistry, Universidad de Chile. [4]Disciplinary Program of Immunology, Institute of Biomedical Sciences, Faculty of Medicine, Universidad de Chile. [5]Medical Research Council Centre for Medical Mycology, University of Exeter, Exeter, United Kingdom.

Hemocyanins are huge and complex glycoproteins used in experimental cancer therapies and vaccines as non-specific and non-toxic adjuvants and immunomodulators. These macroproteins can interact with innate immunity receptors such as C-type lectin receptors (CLRs) and Toll-like receptors (TLRs) on antigen-presenting cells (APCs), generating a robust bias towards a Th1 immune response. The interaction of glycosylated residues of hemocyanins with these receptors allows endocytosis of these glycoproteins, which are then degraded into peptides and loaded onto class II major histocompatibility complex molecules (MHC-II), activating $CD4^+$ T lymphocytes.
This study aims to identify immunodominant epitopes from molluscan hemocyanins for human MHC-II (HLA-II). We focused on examining hemocyanin from the Californian limpet (*Megathura crenulata*, KLH), the gold standard for biomedical applications, and from the Chilean and Peruvian Loco (*Concholepas concholepas*, CCH), which have similar immunogenic properties to KLH but are more stable and soluble.
Epitope prediction software Epibase® and MHC-II Binding Prediction Tool were used, with the available peptide sequences from CCH and KLH2 (gene of the subunit 2 of KLH). Putative epitopes of 15 amino acids with high affinity (Kd<0,1 µM) for at least three HLA-

DRB1 allotypes were selected as candidates, and their immunogenicity and population coverage were analyzed. Four peptides from CCH and two from KLH2 were synthesized, and ongoing experiments are evaluating their immunostimulatory properties using human and mouse APC cell lines.
We expect these individually or pooled peptides to have immunomodulatory effects, and provide a solution to dependence on natural sources of KLH and CCH.
Funding: FONDECYT 1201600 (MIB), ANID 21210946 (MLS), FONDECYT 1231853 (FEG).

26. Potential Health Benefits of Amalaki (*Phyllanthus Emblica*) and Its Role in colon Cancer Risk Reduction. Sergio Bustamante (sebustamante@udec.cl), Jean Pierre Kopplin, Stefania Gonzalez, Soraya Gutierrez, Ariel Castro, Violeta Morin. Department of Biochemistry and Molecular Biology, Faculty of Biological Sciences, Universidad de Concepción.

Introduction. Cancer is one of the leading causes of global mortality, as highlighted by the World Health Organization. Recent research has shown that dietary patterns impact the development of certain types of cancer. Diets rich in red meat and processed foods are linked to increased cancer risks, while diets high in fiber, fruits, and vegetables offer potential protective benefits against the disease. This correlation has heightened interest in natural compounds such as *Phyllanthus emblica*, or Amalaki, known for its health benefits. Amalaki, rich in ascorbic acid, vitamin C, and antioxidant polyphenols, has shown promising results in treating degenerative conditions, cardiovascular issues, and cancer. Amalaki has been used for centuries in India's traditional medicine system, known as Ayurveda.

Materials and Methods. Compounds in the *Phyllanthus emblica* extract were identified using HPLC. Concurrently, the interaction between the extract's components, described in the literature, and the Cdc25 protein, which has been reported to be inhibited by Amalaki, was explored. Additionally, cell viability using crystal violet reagent, cell proliferation using bromodeoxyuridine incorporation, and cell migration were evaluated in the colorectal cancer cell lines cell line, CCD 841 CoN and SW-480 subjected to different concentrations of Amalaki.

Results. The extract's analysis by HPLC-DAD detected a peak in the standard retention time of pyrogallol, indicating this phenolic compound's presence. Therefore, we performed molecular docking and dynamic simulations using Autodock Vina and NAMD software, showing that pyrogallol can interact with the Cdc25 protein. Additionally, we showed that the extract significantly decreased the viability, proliferation, and migration of colorectal cancer cells compared to the normal colon cell line, CCD 841 CoN.

Discussion. This study's findings indicate that pyrogallol may be the active compound in the extract. They underscore Amalaki's potential as a modulator of cellular behavior, shedding light on its therapeutic significance in cancer treatment.

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27. The role of MUL1 in vascular dysfunction in an angiotensin II-induced, ROS-NF-kB-mediated model of arterial hypertension. Ximena Calle[1,2] (ximecalle95@gmail.com), Brenda Becerra[2], Valeria Garrido[2] Angélica Ortega-Muñoz[2], Allan Peñaloza, Francisco Pino De la Fuente[2], Mayarling F Troncoso[2,3], Claudia Muñoz[2], Alejandra Hernández[2], Francisca Valenzuela[2], David Silva[1], Alejandra Guerrero-Moncayo[1], Bernardo Krause, Sergio Lavandero[1,4]. [1]Institute of Health Sciences, University of O'Higgins, Rancagua, Chile. [2]Advanced Center for Chronic Diseases (ACCDiS), Facultad de Ciencias Químicas y Farmacéuticas & Facultad Medicina, Universidad de Chile, Santiago, Chile. [3]School of Medical Technology, Faculty of Medicine, University of Chile, Santiago, Chile. [4]Cardiology Division, University of Texas Southwestern Medical Center, Dallas, Texas, USA

Background: Cardiovascular disease (CVD) is the leading cause of death worldwide and among cardiovascular diseases include hypertension, heart failure, stroke, etc. These pathologies result in maladaptive processes such as changes in vascular wall thickness and lumen diameter known as cardiovascular remodelling. Angiotensin II (Ang II), in the vasculature, induces contraction, cell growth, migration and differentiation, profibrotic and promotes endothelial dysfunction and structural remodeling.
Objective: to study whether MUL1 deficiency prevents VSMC dedifferentiation in vitro and vascular dysfunction in an in vivo angiotensin II model in MUL1 knockout mice. Therefore, MUL1, a mitochondrial and multifunctional protein involved in inflammation and cell growth processes could be a novel therapeutic strategy to lead to regression or prevention of arterial remodeling by producing vascular protection.
Methods: Angiotensin II-induced hypertension model for 14 days at a dose of 1000 ng/kg/min in knock out, MUL1 heterozygous and wildtype animals (CICUA 24775-CQyF-UCH). **Results:** A ChIP Seq analysis was performed in a rat and human model where several studies were identified showing this possible binding of the RELA subunit of NF-κB and BRD4, which is found in the active dimerized form of NF-κB and helps to activate the expression of genes that NF-κB regulates. Where pic values of MACS2 generated within 10 Kb bases of the transcription start site (TSS) are represented. TSS is the site where the putative promoter region of MUL1 is located, and MACS2 serves to identify the sites of a transcription factor; this value captures the influence of genome complexity to assess the importance of Chip-enriched regions, the higher the value means that the factor is located at the non-randomly given site. On the other hand, in the in vivo model a significant increase in heart and artery MUL1 as measured by immunohistochemistry was determined.
Conclusion: MUL1 is therefore involved in cardiovascular diseases specifically in hypertension but its mechanism of regulation and activation is unknown and therefore important to elucidate.
Funding: FONDECYT 1200490 (SL), 1240443 (SL), FONDAP 1523A0008 (SL) and ANID Postdoctorado 3240620, Chile.

28. Unravelling Metastatic Breast Cancer Dynamics: Insights into Rab27-Mediated Extracellular Vesicle Release and Mitochondrial DNA Packaging. America Campos-Gonzalez [1], Jasmine Peters [1], Michalis Gounis [1], Louise Mitchell [1], Celina Shen[2], Christina G.Towers[2], Cassie Clarke [1], Jim C.Norman[1]. [1]Cancer Research UK Scotland Institute, Garscube Estate,Switchback Road,Glasgow,UK. [2]Salk Institute for Biological Studies, La Jolla, CA, USA.

Introduction: Despite treatment advances, metastatic breast cancer remains a leading cause of female mortality. Understanding breast tumour cell signalling and its variation in metastatic environments is crucial for better treatments. The Rab family of small GTPases, which regulate vesicle trafficking, are implicated in breast cancer progression and metastasis. Recent research from our laboratory has revealed that Rab27 regulates the release of EVs containing mitochondrial DNA(mtDNA) via a PINK-dependent mechanism. However, the specific roles of Rab27 isoforms in mitochondrial-derived vesicle(MDV) formation and mtDNA abundance in EVs remain unclear, particularly whether these roles differ between primary breast tumours and their metastases.

Materials and Methods: To address this, we derived several isogenic cell lines from a MMTV-PyMT mouse model of mammary carcinoma, representing primary tumours and corresponding lung metastases. Using CRISPR-CAS9 technology, we generated Rab27a and Rab27b knockouts in these cell lines.

Results: We show that Rab27 deletion reduces MDV formation and the release of mtDNA-containing EVs from primary tumour-derived cells. Conversely, metastasis-derived cells exhibit increased EV release compared to their primary tumour counterparts, with diminished mtDNA content and decreased MDV formation. Notably, EV release from metastatic breast cancer cells is independent of Rab27s, relying instead on sphingomyelinase-2 activity, albeit promoted by Rab27 deletion, particularly Rab27b.

Discussion: These findings suggest a shift in EV production pathways as mammary cancer cells colonize the lung, transitioning from Rab27-dependent/sphingomyelinase-independent to Rab27- independent/sphingomyelinase-dependent pathways. Understanding these dynamics could offer insights into novel therapeutic targets and strategies to mitigate metastatic breast cancer progression.

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29. Genome-wide identification of the family U-box genes in *Actinidia deliciosa* and functional analysis under salinity stress. Emerson Clovis Carrasco-Lozano[1], Samuel Parra[1], Francisco Correa[2], Christian Gonzalez-Calquin[1], Leticia Amaza[1], Michael Handford[1] and Claudia Stange[1]. [1]Plant Molecular Biology Centre, Department of Biology, Faculty of Sciences, Universidad de Chile, Santiago, Chile. [2]Instituto de Investigaciones Agropecuarias (INIA Rayentué), Rengo, Chile

Introduction: Salinity is the most important abiotic stress that reduces crop yields worldwide. Many species are susceptible to salinity, including *Actinidia deliciosa* cv.

Hayward, which is also an economically important species in our country. Plants have developed tolerance mechanisms to salinity at the physiological, biochemical, and molecular levels. In this regard, they possess ubiquitination mechanisms where E3 ligases target proteins for degradation by the 26S proteasome to maintain protein homeostasis. The activity of E3 ligases as negative regulators to abiotic stress has been reported in many plants; however, it is unknown in *A. deliciosa* cv. Hayward. Therefore, this study aimed to identify U-box type E3 ubiquitin ligase family genes and evaluate their function as negative regulators in response to salinity stress.
Methods: By means of bioinformatics, expression analysis under salinity, subcellular localization and the functional characterization of *N. tabacum* that overexpress *AdPUB11* we aimed to identify U-box type E3 ubiquitin ligase family genes and evaluate the function of *AdPUB11* as negative regulators in response to salinity stress.
Results: *A. deliciosa* cv. Hayward genome contains 88 U-box type E3 ubiquitin ligase genes distributed across all chromosomes and classified into eight groups, like *Arabidopsis thaliana*. Among these genes, *AdPUB11* stands out showing a reduced expression level in leaves. We determined its expression in roots subjected to 200 mM NaCl, showing an induction at 48 hours after salt treatment compared to the wild type. We also determined that AdPUB11:GFP localized to the plasma membrane. Furthermore, lines overexpressing the *AdPUB11* gene in *N. tabacum* exhibited susceptibility to salinity stress under *in vitro* conditions, displaying a smaller leaf area and root length compared to the wild type.
Discussion: Together, these results indicate that *AdPUB11* negatively regulates salinity stress responses in *A. deliciosa* cv. Hayward.
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30. Spontaneous condensation of prebiotic amino acids can produce peptides that self-assemble into catalytically active amyloids. Daniel Carrillo[1] (graef.stift@gmail.com), Cristián Tirapegui[2], Rodrigo Díaz-Espinoza[1]. [1]Laboratorio de Biofísica Molecular, Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile. [2]Universidad Autónoma de Chile, Instituto de Ciencias Aplicadas, El Llano Subercaseaux 2801, San Miguel, Santiago, Chile.

Introduction: All modern living organisms rely on highly complex molecules for structure and function. However, the chemistry preceding the origin of life had to be simple. Ribonucleic acids can encode hereditary and catalytic power in a single molecule; however, they are unstable, difficult to synthesize abiotically, and require large sizes to achieve biological functions. Peptides on contrast are simple and have diverse and feasible prebiotic routes for their synthesis, but they are unstable and poorly active. Recently, an alternative

path has emerged in which the assembly of peptides into highly stable intermolecular arrays can produce emergent behavior. Condensation of amino acids into peptides under prebiotic conditions form intermolecular assemblies called amyloids. Here, we show that these assemblies can also be catalytic.
Materials and Methods: Homopeptides were prepared by condensation of prebiotic amino acids using 1,1-carbonyldiimidazole (CDI) as a condensing agent, followed by days-long incubation to promote self-assembly into amyloids. These aggregates were analyzed by fluorescence of the amyloid-specific probe Thioflavin-T (Th-T) and transmission electron microscopy (TEM). The catalytic activity of the assemblies was assayed by measuring the catalyzed hydrolysis of adenosine triphosphate (ATP) at pH 8 in presence of manganese ions (Mn^{+2}). Products (free phosphate anions) were determined by colorimetric spectrophotometry.
Results: The presence of amyloids upon condensation of certain prebiotic amino acids was estimated by a specific increase of Th-T fluorescence signal, and formation of whitish pellets could be visibly registered. Initial velocities of ATP hydrolysis above established minimum values of uncondensed amino acids and post-condensation imidazole product were obtained for polyvaline, polyalanine, polyleucine, polyisoleucine and polyproline. These results were not observed for polyglycine, polyglutamate, polyserine and polythreonine.
Discussion: We can establish for the first time a possible enzyme-like activity of amyloids assembled with certain prebiotic amino acids.
Acknowledgment: FONDECYT 1211821 and DICYT PS530 Code.022343DE_Postdoc.

31. Altered epigenetic regulation of ZNF560 gene expression in schizophrenia-derived hiPSC. Bárbara S. Casas (barbara.s.casas@gmail.com)[1,2], Elvis Acevedo[2], Claudio Letelier[2], Víctor Pola-Véliz[2], Verónica Palma[1] & Martín Montecino[2]. [1]Laboratorio de Células Troncales y Biología del Desarrollo, Facultad de Ciencias. Universidad de Chile, Chile. [2]Laboratorio de Regulación Génica, Instituto de Ciencias Biomédicas. Universidad Andrés Bello, Chile.

Introduction: Zinc finger proteins (ZNFs) are a large family of nucleic acid-binding proteins, with more than 500 members encoded along the human genome. ZNF binding to DNA can regulate gene transcription, which plays an important role during development as well as in the tissue physiology of mammals. Moreover, altered expression and malfunction of ZNFs have been associated with several neurological conditions, including Parkinson's disease, Alzheimer's disease, epilepsy, and schizophrenia. Here, we report a dysregulation in the expression of a novel *ZNF* gene in hiPSC derived from schizophrenia patients (SZ).
Materials and methods: Differential global mRNA expression between schizophrenia (SZ)-(N=4) and healthy control (N=3)-derived hiPSCs, was evaluated through RNA sequencing. DNA methylation profiles were determined by Sanger sequencing after bisulfite cytosine conversion.
Results: Transcriptomic analysis assessing the differential mRNA expression profile

between HC- and SZ-derived hiPSC revealed a significant upregulation of *ZNF560* mRNA in SZ samples. As a genomic database analysis predicted the presence of a CpG island at the *ZNF560* gene promoter, DNA methylation along this region was determined by sequencing of bisulfite-converted cytosines. It was found that the percentage of DNA methylation at the *ZNF560* gene promoter was markedly reduced in hiPSC derived from SZ patients.
Discussion: Together, these results indicate that increased expression of *ZNF560* in SZ hiPSC correlates with reduced CpG methylation at the *ZNF560* promoter. This implies that a DNA demethylation-mediated mechanism can be associated with the altered *ZNF560* gene expression detected in SZ pathophysiology, hence representing a putative early marker of this disease.
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32. Minimalistic enzymes mimicking lacasse activity against a phenolic compound.
Claudio Castillo-Cáceres (cla.castillo.c@gmail.com), Rodrigo Díaz. Universidad de Santiago de Chile.

Introduction: Enzymes are highly complex biomolecules with great biological and technological relevance. Laccases are among the most widely used enzymes in the industry , thanks to its copper (Cu^{2+})-mediated oxidoreductase activity. They catalyze the oxidation of a wide range of toxic phenolic compounds, which makes them suitable for diverse biotechnological applications. However, implementing these and other biocatalysts in the industry typically brings important challenges such as large-scale expression and purification of the recombinant proteins and meeting stability requirements in the intended application site. Thus, a main challenge in modern enzymology is the search for enzymes that are easier to produce and more resistant and flexible to different environmental conditions. In this work, we show how small peptides can produce intermolecular assemblies that are competent in simulating and imitating the catalytic properties of laccases, while overcoming their natural stability and chemical limitations. Using rational design, we generated two peptides that intercalated histidine with nonpolar residues, allowing the assembly to coordinate Cu^{+2}, mimicking the active site of laccases.
Materials and Methods: The activity was measured by the chromogenic reaction of 2.4 DP with 4-AAP (aminoantipyrine) at 510 nm, using a 96-well UV transparent plate.
Results: Different histidine-containing peptides spontaneously assembled into amyloid-like structures that bind the amyloid-specific probe thioflavin T (Th-T). The assemblies were active against a canonical phenolic pollutant (2,4-Dichlorophenol), catalyzing its oxidation in a similar fashion as laccase. Peptides 1 and 2 showed the highest specific activities, averaging 3.23 $\mu M\ min^{-1}\ mg^{-1}$ and 2.36 $\mu M\ min^{-1}\ mg^{-1}$, respectively, surpassing a model thermostable laccase (ID17) that exhibited 1.2 $\mu M\ min^{-1}\ mg^{-1}$. The peptide assemblies were active in buffered solutions as well in plain tap water. The activity was retained even upon exposure to high temperature.

Conclusion: Self-assembled peptides can exhibit oxidoreductase activity that can mimic laccase under different industrially relevant conditions. The results demonstrate that small peptides can be promising candidates in their assembled state as future minimalistic biocatalysts.
Acknowledgment: Proyecto ANID Explorador 13220108.

33. Microdissection of CA1 for the evaluation of chronic stress on local transcriptome in neuropil of dorsal hippocampus in rats. J. Catalán-Casanellas (julia.catalan@ug.uchile.cl), J.P. Silva, Olave F.A. W.A. Corrales, Alarcón M., T. A. Guarnieri., Gonzalez P. and J.L.Fiedler. Laboratory of Neuroplasticity and Neurogenetics, Department of Biochemistry and Molecular Biology. Faculty of Chemical and Pharmaceutical Sciences. Universidad de Chile,

Introduction: Local protein translation plays a crucial role in dendrite and axon outgrowth, synapse formation and maturation which are events required for neuron plasticity of the brain. It is well described that neuroplasticity related processes are severely affected in chronic stress models in rats. The CA1 region of the dorsal hippocampus is characterized by expressing several forms of plasticity that require local protein translations. Recent studies demonstrate differential transcriptomic profiles between dendrites-rich (neuropil) and cell somas-rich (somata) layers under basal conditions. However, the effects of stress on these profiles remain unclear. This study aims to methodological characterization to obtain a specific transcriptomic profile of the neuropil and somata layer under basal conditions and chronic restraint stress.
Materials and methods: Adult female Sprague Dawley rats were subjected to chronic restraint stress to obtain hippocampal slices (500 um) at the coordinates Paxinos from Bregma -2,3 to -4.0 for microdissection of dendrite-rich and soma-rich layer in CA1 region. This was confirmed by quantifying nuclear (NeuN) and dendritic (Psd95 and CamkIIa) markers in both layers by RT-qPCR and Western Blot. Finally, RNA integrity and purity were assessed by Qubit and Fragment Analyzer to check suitable samples for directRNA sequencing by Nanopore.
Results: Successful tissue microdissection was confirmed by an increase of Psd95 and CamkIIa transcript levels in neuropil and increase of NeuN protein in the somata layer. On the other hand, high-quality RNA (260/280 ≈ 2.01; R.Q.N = 9.1) was obtained suitable for sequencing, resulting in a transcriptomic profile with 10.956 mRNA differentially expressed in neuropil under chronic stress.
Discussion: Ontological analysis of differentially expressed transcripts reveal functions related to protein turnover and morphological changes. These findings may explain both changes in CA1 apical dendrites arborization and improvement of hippocampus-dependent spatial memory under chronic stress.
Acknowledgement: FONDECYT Regular 1230471.

34. A cell-free platform for low-cost and on site production of biological reagents. Séverine Cazaux (smcazaux@uc.cl)[1,5], Valentina Ferrando[1,5], Anibal Arce[2], Justin Vigar[4], Quinn Matthews[4], Severino Jefferson Ribeiro da Silva[4], Keith Pardee[4], Alejandro Rojas-Fernández[3], Fernán Federici[1,5]. [1]Instituto de Ingeniería Biológica y Médica, Pontificia Universidad Católica de Chile. [2]Department of Chemical and Biological Engineering, Northwestern University. [3]Facultad de Medicina, Universidad Austral de Chile. [4]Leslie Dan Faculty of Pharmacy, University of Toronto. [5]Instituto de Biología Integrativa.

Introduction: The prototyping and manufacturing of biological reagents such as molecular biosensors, enzymes or diagnostic tools usually involve the centralized production and distribution from countries located in the Global North, thus making their access difficult for medium and low-income countries. In recent years, the use of cell-free based technology has enabled the local production of biological reagents. Compared to commercially available cell-free alternatives, based on reconstituted Tx-Tl systems, lysates can reduce the costs by two orders of magnitude. Here we report the use of locally-produced lysates for the low-cost and on site production of molecular reagents such as toehold switches, enzymes and nanobody-based biosensors.
Results: Fresh and freeze-dried cell-free extracts were used to express a SARS-CoV-2 specific- toehold switch, enzymes for RT-LAMP reactions (Bst-LF and MMLV) and a split T7-based fusion biosensor using alpaca-derived nanobodies. The production of enzymes was performed in a remote area of Tierra del Fuego, (Karukinka park), using open source hardware such as a 3D-printed hand centrifuge and a shaker. Bst and MMLV enzymes were successfully used for the amplification of a synthetic target for Malaria and SARS-CoV-2. The toehold switch was successfully activated by a synthetic target from SARS-CoV-2 using both fresh and freeze-dried extracts, displaying a colorimetric readout for concentrations above 1.6uM of ssDNA or 300nM of RNA. Each fragment of the split T7-biosensor was produced in lysates with different efficiencies.
Discussion: These results suggest opportunities for the decentralized and on site production of biological reagents using cell-free systems based on lysates.
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35. The cell-based TARGET assay to identify directly regulated genes of transcription factors at genome-scale in *Solanum lycopersicum* (Tomato). Ariel Cerda[1,2] (ariel.cerda.rojas@gmail.com), Mauricio Arias[1,2], Sebastián Contreras[1,2], Elena Vidal[2,3], José Miguel Álvarez[1,2]. [1]Centro de Biotecnología Vegetal, Universidad Andrés Bello, Chile. [2]Agencia Nacional de Investigación y Desarrollo – Millenium Science Initiative Program, Millenium Institute for Integrative Biology (iBio), Santiago, Chile. [3]Centro de Genómica y Bioinformática, Facultad de Ciencias, Ingeniería y Tecnología, Universidad Mayor, Santiago, Chile.

Introduction: Identifying the direct targets of transcription factors (TFs) is crucial for understanding plant gene regulation related to growth, development, and stress responses. We established the TARGET (Transient Assay Reporting Genome-wide Effects of Transcription factors) assay in *S. lycopersicum* (tomato) to study ABF5, a key TF for drought response. Our work enhances our understanding of drought tolerance by revealing ABF5's gene regulatory network and demonstrating the efficacy of the TARGET system in tomatoes, a relevant crop for global agriculture. The TARGET system for ABF5 allows for rapid and effective identification of directly induced or repressed genes that participate in tomato responses to drought.
Materials and Methods: Protoplasts from 'Money Maker' tomato cotyledons were PEG-transfected with a vector to overexpress ABF5 fused to a glucocorticoid receptor (GR). Dexamethasone was applied to facilitate its nuclear translocation. GFP-expressing cells were isolated using fluorescence-activated cell sorting (FACS), and RNA was extracted to prepare sequencing libraries. Differentially expressed genes and Gene Ontology (GO) enrichment analyses were conducted to identify over- and under-represented GO terms, elucidating the biological processes, cellular components, and molecular functions involved.
Results: Tomato cotyledon protoplasts were successfully transformed, and GFP tagging facilitated the precise isolation of transformed cells by FACS. Analysis of the transcriptome response revealed that ABF5 directly regulates 2,987 genes, with 1,711 upregulated and 1,276 downregulated. Known TFs in the drought signaling pathway are downstream of ABF5. In addition, ABF5 directly controls biological processes related to response to water deprivation, hormone-mediated signaling pathways, and response to abscisic acid.
Discussion: The TARGET assay in tomatoes represents a significant methodological advancement. It effectively maps TF targets, eliminating the need for stable transformations. Our study reveals how ABF5 modulates plant responses to drought and provides insights that support the development of biotechnological strategies to enhance agricultural sustainability under stress conditions.
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36. bZIP25 as a negative regulator in endocytic trafficking modulates the response to salt stress in *Arabidopsis thaliana.* Damián Cifuentes[1] (damiancifuentesaguilar@gmail.com), Lorena Pizarro[2] and Lorena Norambuena[1]. [1]Plant Molecular Biology Centre, Department of Biology at Faculty of Sciences, Universidad de Chile. [2]Instituto de Ciencias Agroalimentarias, Animales y Ambientales. Universidad de O´Higgins, San Fernando, Chile.

The endomembrane system comprises a complex network of functionally connected compartments. In our laboratory, we have found that the leucine zipper transcription factor

bZIP25 is involved in endocytosis in Arabidopsis. The loss-of-function mutant of *bZIP25* (*bzip25-2*) displays a higher rate of endocytosis, indicating that it regulates negatively the endocytic trafficking. There are three bZIP25 isoforms due to alternative splicing. *bZIP25.1* and *bZIP25.2* are expressed in roots. The expression of *bZIP25.1-GFP* or *bZIP25.2-GFP* isoforms in the mutant background results in slowing down the endocytosis rate up to the level of wild type seedlings. Transcriptional profile reveals several salt (NaCl) stress response genes that are being differentially expressed in the loss of function of *bZIP25.* This includes genes involved on calcium signalling, ROS scavenging and salt stress response which along with the endocytosis phenotype of the mutant driven us to investigate whether bZIP25 would be involved in salt stress response. Therefore, we analysed the phenotype of *bzip25-2* mutant and the mutant lines that express the *bZIP25.1* or *bZIP25.2* isoform under salt stress conditions. *bzip25-2* seedlings were more tolerant to salt treatment, displaying higher survival rate than wild type seedlings. Under salt treatment, endocytic trafficking is induced in wild type seedlings. Nevertheless, the higher rate of endocytosis of *bzip25-2* seedlings was not further induced in response to salt. To evaluate the cellular response to salt stress, the level of ROS was assessed. The mutant *bzip25-2* displayed lower levels of ROS in control condition than wild type; levels that were not induced by salt stress. The unresponsiveness of *bzip25-2* to salt stress on the generation of ROS and induction of endocytosis in response indicates a role of bZIP25 on salt response. The expression of *bZIP25.1-GFP* but not *bZIP25.2-GFP* rescued the salt tolerance phenotype of the loss-of-function mutant, suggesting different roles for bZIP25 isoforms in Arabidopsis roots. Taken together, these results point to bZIP25 as a negative regulator in endocytic trafficking with a role in salt stress response, probably through regulating ROS formation.
Acknowledgment: FONDECYT 1211311 and PhD fellowship “Beca de Doctorado Nacional” ANID.

37. Nitrogen availability impacts phenotypic and transcriptomic responses of *Solanum lycopersicum* to drought. Catalina Cofré-Espinoza[1] (c.cofrespinoza@uandresbello.edu), Tomás C. Moyano[1,2], Luciano Ahumada[1,2], José Miguel Alvarez[1,2]. [1]Centro de Biotecnología Vegetal, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago, Chile. [2]Agencia Nacional de Investigación y Desarrollo – Millenium Science Initiative Program, Millenium Institute for Integrative Biology (iBio), Santiago, Chile.

Introduction: Plants are frequently subjected to multiple signals that must be harmonized to achieve a balance between growth and stress response. Nitrogen (N) and water (W) are crucial factors for plant growth, and their scarcity can significantly hinder the development of crops, ultimately impacting the agricultural sector. While the individual effects N and drought on plants have been widely studied, surprisingly little is known

about how plants respond to their combination. Tomato (*Solanum lycopersicum*), a relevant crop susceptible to both N and W levels, is an ideal model to understand the complex transcriptome and phenotypic responses to these combined stresses, thanks to its well-characterized genomic resources.

Materials and Methods: Tomato plants were divided into four groups, each receiving a different combination of N concentrations and irrigation treatments. The control groups were divided into two categories, with contrasting N concentrations: high N (HN 10 mM) vs. low N (LN 1 mM) and 100% field capacity. The two experimental groups were given 50% field capacity with varying N concentrations. All groups underwent morphological, physiological, and transcriptomic (RNA-seq) examinations in leaf tissue.

Results: We found HN levels produced significant alterations in morphological and physiological parameters related to drought. When subjected to HN levels, leaf turgor decreased, while the density of stomata, a pore that allows water and gas exchanged, increased. Transcriptome analysis revealed a higher number of differentially expressed genes in response to HN versus LN under drought conditions. Importantly, biological processes related to stomata function and drought adaptations are more enriched in HN compared to LN.

Discussion: Our findings reveal a significant impact of N dose on phenotypic and transcriptomic responses to drought. These results indicate that the plant's response to drought is influenced by its nutritional status, balancing growth and stress response according to N levels.

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38. NUAK1 promotes pentose phosphate pathway via hnRNPK-G6PD and favours oxaliplatin chemoresistance. Viviana Coliboro-Dannich (vcoliboro@udec.cl), Alejandro Farías, Luis Espinoza, Mario Palma, Roxana Pincheira[1], Ariel Castro. Laboratorio de Transducción de Señales y Cáncer, Facultad de Ciencias Biológicas, Universidad de Concepción.

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. In this work, we searched for interactors of NUAK1 to define its association with the PPP.
Materials and methods: We used HCT116p53-/- and SW480 colorectal cancer (CRC) cells. NUAK1 interactors were identified by mass spectrometry and verified by immunocytochemistry and co-immunoprecipitation analysis. In vitro kinase assays and immunodetection of phosphorylation residues were conducted. RT-qPCR and RNA immunoprecipitation were used to determine NUAK1's effects on splicing and mRNA interaction. We assessed protein levels using western blot and measured NADPH, ROS, and lipid levels to study the effects on PPP. Finally, we combined the NUAK1 inhibitor HTH-01-015 with oxaliplatin in 3D culture to determine NUAK1's role in chemoresistance.
Results: We found that NUAK1 interacts with hnRNPK in CRC cells. HnRNPK is a ribonucleoprotein that inhibits the splicing of glucose-6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme of PPP. Interestingly, NUAK1 promoted hnRNPK phosphorylation, suggesting that NUAK1 inhibits hnRNPK interaction with its RNA targets. Consistently, NUAK1 promoted G6PD mRNA processing and inhibited hnRNPK-G6PD mRNA interaction. These results correlated with higher levels of G6PD protein, higher NADPH, increased lipid accumulation, and lower ROS levels. In addition, NUAK1 inhibition reduced cell viability when combined with oxaliplatin, suggesting that it is involved in chemoresistance.
Discussion: Our study suggests that NUAK1 promotes the PPP through the hnRNPK-G6PD axis in CRC cells, involving NUAK1 in cancer progression and oxaliplatin chemoresistance.
Acknowledgment: FONDECYT 1201215, 1241771.

39. NUAK1 promotes lipid accumulation through SREBP maturation in colorectal cancer cells. Andrea Concha (mconcha2020@udec.cl), Viviana Coliboro-Dannich, Alejandro Farias, Luis Espinoza-Francine, Roxana Pincheira, Ariel Castro. Signal Transduction and Cancer Laboratory, Biochemistry and Molecular Biology Department, Faculty of Biology Sciences, Universidad de Concepción, Concepción, Chile.

Introduction: Lipid metabolism reprogramming is an important hallmark of cancer progression. SREBP is a master transcription factor in lipid metabolism, and its activation involves intricate mechanisms, including its maturation by proteolysis. A gene ontology analysis based on RNA-seq revealed that NUAK1 positively regulates SREBP's transcriptional control of lipogenesis. NUAK1 is a Ser/Thr kinase overexpressed in several types of cancer and associated with poor prognosis. Interestingly, NUAK1 increased NADPH levels, an important cofactor in lipid biosynthesis, in colorectal cancer cells (CRC). This work aimed to determine whether NUAK1 promotes SREBP maturation and the expression of its target lipogenic genes in CRC.
Materials and methods: FLAG-hNUAK1 was overexpressed in HCT116P53-/- and SW480 CRC cultured in 10% serum or serum-deprived conditions. Forty-eight hours post-transfection, cells were lysed or fractionated into cytoplasm and nucleus for western blotting

analysis. RNA extraction, BODIPY-488 dyeing, and immunocytochemistry were also performed 24h post NUAK1 overexpression in the same conditions.
Results: NUAK1 overexpression increased SREBP maturation and the mRNA levels of ACC, LDLR, and FASN, known SREBP target genes. Accordingly, NUAK1 overexpression induced lipid accumulation in CRC.
Discussion: Our results suggest that NUAK1 is involved in lipid metabolism reprogramming in CRC through increasing SREBP-dependent expression of lipogenic genes.
Acknowledgment: FONDECYT 1201215, 1241771.

40. Analysis of Gene Expression Models in Response to Drought: Comparison between Drought-Adapted and Commercial Tomato. J. Sebastián Contreras-Riquelme (scontreras@ibio.cl)[1,2], Miguel Contreras[1,2], Rachid Sjoberg[1,2], Tomas C. Moyano[1,2] & Jose M. Alvarez[1,2]. [1]Plant Genome Regulation Lab, Centro de Biotecnología Vegetal, Facultad de Ciencias de la Vida, Universidad Andrés Bello. [2]ANID Millenium Institute for Integrative Biology iBio, Chile

Introduction: Drought is the most detrimental abiotic stress for plant growth, affecting key processes such as germination, photosynthesis, and metabolic pathways. With climate change exacerbating water scarcity, understanding plant adaptation to drought is critical. Tomatoes (*Solanum lycopersicum*) face significant challenges under drought conditions, altering gene expression and thus stress responses. However, tomato-related species like *Solanum pennellii*, which thrive in arid environments, offer valuable insights into drought tolerance mechanisms. In this study, we explore gene expression models between both species, focusing on “stress-ready” genes constitutively expressed/repressed that pre-adapt plants to extreme conditions.
Materials and Methods: To establish orthology among genes of *S. lycopersicum* and *S. pennellii*, we conducted a *blastp* analysis. Then, using transcriptomic data of *S. lycopersicum* and *S. pennellii* plants under drought, we perform gene expression quantification through *Kallisto* followed by a differential expression analysis using *DESeq2*. Using this information, we built a decision tree to assign ortholog states, which include shared responses and four cases for "stress-ready” responses to drought. Then, we perform Gene Ontology term enrichment analysis in these groups and finally, we search for key regulators of these responses through motif enrichment and Gene Regulatory Networks (GRN) analysis using *FIMO* and *GENIE3* machine learning predictor.
Results: We found that nearly 35% of orthologs present a “stress-ready” state of gene regulation, with enrichment of function related to Histones and RNA metabolic processes, with ERF Transcription Factor family as potential regulators of “stress-ready" in *S. pennellii*.
Discussion: Desert-adapted tomato plants exhibit pre-emptive mechanisms to maintain a "primed" state, allowing them to respond rapidly and effectively to stress. This involves the upregulation of specific genes and pathways even before the onset of stress, enabling these plants to withstand drought conditions better than non-adapted tomatoes. The promoters of

such genes are enriched in specific motifs that TF recognizes, suggesting variation in regulatory sequences is important for plant adaptation to drought.
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41. Exploring regulatory effects of stress and corticosterone on the m^{6}A landscape of the dorsal hippocampus in male and female rats. W. A. Corrales[1] (wcorrales@ug.uchile.cl), J. P. Silva[1], J. T. Lee[2], J.L. Fiedler[1]. [1]Laboratory of Neuroplasticity and Neurogenetics, Department of Biochemistry and Molecular Biology, Faculty of Chemical and Pharmaceutical Sciences Universidad de Chile, Santiago, Chile. [2]Department of Molecular Biology, Massachusetts General Hospital; Department of Genetics, Harvard Medical School, Boston, MA, USA.

Introduction: The dorsal hippocampus plays a critical role in learning and memory, and it is an important structure in neuropsychiatric disorders. Recent research has drawn attention to RNA modifications (epitranscriptome) such as N6-methyladenosine (m^{6}A) that can significantly impact RNA metabolism and subsequent cellular processes. Considering that neuroplastic responses to stress in the hippocampus may be affected by both sex and m^{6}A dynamics, our goal was to explore the effect of chronic stress on male and female rat dorsal hippocampus and to determine whether changes in m^{6}A are influenced by the main stress hormone corticosterone.
Materials and Methods: We used a depressive-like paradigm (chronic restraint stress, 2.5 h per day for 14 days) on adult male and female Sprague-Dawley rats, aiming to evaluate stress and sex-biased changes in the m^{6}A machinery levels and m^{6}A epitranscriptomic profiles of the dorsal hippocampus through bulk RNA-seq and Nanopore directRNA-seq. Additionally, we tested whether corticosterone could elicit changes in the m^{6}A machinery transcript levels in a mouse hippocampal-derived cell line (HT22).
Results: Bulk RNA-seq unveiled stress and sex-biased transcripts in the dorsal hippocampus involved in axonal guidance, GABAergic, glutamatergic and serotoninergic synapses, as well as genes that encode the m^{6}A machinery (*Mettl3*, *Alkbh5*, *Ythdf1/2*). In addition, m^{6}A-aware basecalling of Nanopore directRNA-seq data allowed us to identify 37 m^{6}A sites differentially modified depending on sex and stress. Functional enrichment analysis of the m^{6}A-modified transcripts showed a potential influence of this mark on energy metabolism, glutamate signaling, and pathways related to neuropsychiatric disorders. Finally, corticosterone treatment of HT22 cells induced an increase in the transcript levels of the m^{6}A writer complex main components (*Mettl3*, *Mettl14*).
Discussion: These findings suggest that chronic stress and sex may impact the dorsal hippocampus function on memory and learning through m^{6}A dynamics, which could be influenced by corticosterone-dependent and independent mechanisms.

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42. Comparison of conformational dynamics of ThiD-HMPPK enzymes from a thermophilic and a mesophilic lineage. Isaac Cortés-Rubilar (isaac.cortes@ug.uchile.cl), Myriam Pérez, Nikolas Knoop-Siegel, Exequiel Medina, Victor Castro-Fernández. Laboratorio de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Chile, Santiago, Chile.

Thermophilic proteins are stable at temperatures higher than 60°C due to adaptations that allow them to perform their function at this temperature. In general, among these adaptations, it has been described as having lower conformational dynamics at room temperature than a mesophilic homologous protein. However, the characteristics that determine thermostability are not ubiquitous since evolutionary history can modulate the adaptive strategies. In addition, the relationship between increased thermostability and decreased conformational dynamics of thermophilic proteins has recently been questioned. In this work, we aim to compare the conformational dynamics of two ancestral enzymes of the ThiD-HMPPK family, one from a mesophilic evolutionary lineage of bacteria (Enterobacteriales, ancEn, Tm=59 °C) and another from a thermophilic lineage (Thermus, ancTh, Tm=85 °C), through single-molecule fluorescence anisotropy (smFA) and accelerated molecular dynamics (aMD) simulations. Both proteins, ancTh, and ancEn, were derivatized with Bodipy dye to measure smFA to compare the local structural flexibility in a catalytic loop. Additionally, Gaussian aMD (GaMD) simulations were performed to analyze the conformational dynamics of both proteins. GaMD simulations showed overall lower conformational dynamics for the thermophilic (ancTh) regarding the mesophilic protein at 300 K. However, specific regions of the thermophilic protein appear to adopt a greater variety of conformations than the mesophilic protein. Consistently, smFA experiments showed increased local flexibility in the catalytic loop for ancTh at room temperature, loop that also shows higher dynamics in GaMD simulations. Taken together, these experiments suggest that ancTh has overall lower global conformational dynamics than ancEn at room temperature, but specific catalytic regions do not follow this behavior.
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43. Role of NCLX in Angiotensin II-Induced Cardiac Hypertrophy. Wendy Lai Li[1,2,3], Mayarling Francisca Troncoso[1,2,3], Pablo Cruz[3], Sergio Lavandero[1,2], Marioly Müller[1,2,3]. [1]Laboratorio de Transducción de Señales Moleculares, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile. [2]Advanced Center of Chronic Diseases (ACCDiS), Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile. [3]Departamento de Tecnología Médica, Facultad de Medicina, Universidad de Chile.

Introduction: Cardiac hypertrophy is a pathological condition characterized by an increase

in cell size that might lead to severe cardiovascular diseases and sudden death. In heart failure models, NCLX, the mitochondrial Na^+/Ca^{2+} exchanger, is crucial for Ca^{2+} homeostasis. In this study, we explore the role of NCLX in Angiotensin II (Ang II)- induced cardiac hypertrophy. We hypothesize that inhibition or decreased expression of NCLX leads to mitochondrial Ca^{2+} overload and subsequently enhances the (Ang II)-induced cardiac hypertrophy.
Materials and Methods: We treated neonatal rat ventricle cardiomyocyte cultures with 100 nM Ang II at different times. Changes in cell size, hypertrophy markers, and NCLX expression were determined through immunoblots, RT-qPCR, and immunofluorescences. We performed calcium imaging using Fluo-4-AM and Rhod-2-AM to evaluate the cytoplasmic and mitochondrial Ca^{2+} signals. We used the NCLX inhibitor, CGP-37157, to investigate the contribution of NCLX in cardiac hypertrophy and Ca^{2+} signals.
Results: Ang II treatment significantly increased cardiomyocyte size, indicating hypertrophy. The 24-hour Ang-II treatment induced a significant reduction in NCLX expression. Additionally, both Ang-II and CGP-37157 treatments resulted in increased mitochondrial Ca^{2+} release. Co- incubation with Ang-II and CGP-37157 further enhanced cardiomyocyte hypertrophy without affecting cell viability. These findings suggest that NCLX activity contributes to the pathogenic mechanism in the (Ang II)-induced cardiac hypertrophy model.
Discussion: These findings suggest that NCLX plays a critical role in regulating mitochondrial Ca^{2+} levels during (Ang II)-induced cardiac hypertrophy. The decrease in NCLX expression may contribute to mitochondrial dysfunction and, subsequently, cardiac dysfunction, highlighting NCLX activity as a potential therapeutic target.
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44. Characterization and functionality of HBx protein isoforms and the ORF6 protein of the Hepatitis B virus. Perla Cruz Riquelme (pcruzriquelme@gmail.com), Scarleth Larrain, Constanza Ortiz, Rodrigo Villanueva, Alejandra Loyola. Faculty of Medicine and Sciences, Universidad San Sebastián, Santiago de Chile, Laboratory of Epigenetics and Chromatin, Fundación Ciencia & Vida.

Introduction: Hepatitis B virus (HBV) is a hepatotropic virus whose genome consists of partially double-stranded circular DNA. HBV encodes a single small regulatory protein called X protein or HBx, which has three isoforms: XF, XM, and XS, as well as the hypothetical viral protein called ORF6, whose reading frame overlaps with the HBx reading frame but in the opposite direction. These proteins have different properties that affect the regulation of the cell cycle and cellular functioning. Based on this, this study aims to characterize these proteins in hepatocarcinoma stably transfected cells.
Materials and Methods: This research utilized HepG2 cell transfected with GFP fused with XF, XM, XS, and ORF6 isoforms. The cells were analyzed by microscopy,

immunoprecipitation, flow cytometry, mass spectrometry, mitochondrial extraction and digestion, clonogenic assays and cell proliferation assays.
Results: Differences in the analyzed properties between HepG2 cells and their isoforms were demonstrated. Additionally, the mitochondrial localization of the XM protein was detected.
Discussion: The results of this investigation provide information on the characterization and functionality of each HBx protein.
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45. The evolution of nitrate sensing in land plants. Del Rosario-Chávarri, Jorge (jldelrosario@uc.cl); Gutiérrez, Rodrigo A. Millennium Institute for Integrative Biology (iBio). Millennium Institute Center for Genome Regulation (CRG). Institute of Ecology and Biodiversity (IEB). Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

Introduction: Nitrate is one of the main sources of inorganic nitrogen for land plants, acting as a signal molecule that modulates many aspects of growth and development. Nitrate sensors have been identified and characterized in *Arabidopsis thaliana* and a few other species. The overall relevance of these nitrate sensors for land plants in general remains unclear. Current phylogenetic studies mainly focus on angiosperms and do not consider other representative land plant lineages. Our work aims to trace the evolutionary history of nitrate sensing across different land plant lineages, focusing on their phylogenetic relationships, protein domain architectural organization, and distinctive motifs.
Materials and Methods: Seventeen plant species, from Charophytes to Angiosperms, were selected. Sequences from the two nitrate sensor systems identified to date (transceptor and transcription factor) were used to download related family proteins from the Ensembl Plant database. Phylogenetic analyses were carried out using Bayesian and maximum likelihood methods, followed by a posterior reconciliation analysis. Protein domain architecture and specific motif enrichment were determined and compared.
Results: There is a significant expansion in nitrate sensor-related family members from charophytes to angiosperms. Phylogenetic relationships showed that described nitrate sensors originated from a posterior duplication event from basal land plant lineage divergences. Additionally, members of phylogenetic subgroups that include described nitrate sensor show similarities in architectural characteristics, distinctive motifs, and nitrate interaction regions.
Discussion: As duplication may lead to pseudogenization, function compensation, or neofunctionalization, it is unclear whether nitrate sensor activity was present in the common ancestor of land plants. Nonetheless, architectural protein domains and specific region similarities suggest that the studied proteins may have developed a specific nitrate sensing function through modifications not necessarily present in the common ancestor.

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46. Molecular memory in response to fluctuations in nitrate availability in *Arabidopsis thaliana.* Laura D. Delgado (lddelgado@uc.cl) and Rodrigo A. Gutiérrez. Millennium Institute for Integrative Biology. Millennium Institute Center for Genome Regulation Institute of Ecology and Biodiversity. Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

Introduction: Plants integrate environmental cues and respond in an optimized-manner to future environmental challenges depending on their past experience-a phenomenon known as “memory”. While extensively studied in various (a)biotic-stress contexts, its role in response to fluctuating nutrient-availability remains unexplored. Here, we investigated whether *Arabidopsis thaliana* plants have a “nutritional-memory” that modulates their response to fluctuating nitrate-availability, the primary N-source in aerobic soils.
Methods: We evaluated whether plant response to nitrate-treatments is conditioned by previous N-exposures. We assessed morphological (growth and developmental parameters) and molecular phenotypes (RNA-seq) to evaluate the impact of nutritional-memory and propose candidate regulatory factors mediating the process.
Results: Primed plants exhibited an improved root system architecture than unprimed plants after the triggering-stimulus. No significant differences in total N-content were observed; however, the C/N-ratio varied among treatments. RNA-seq analysis demonstrates that, during recovery phase, primed plants exhibit higher transcript levels of prototypical nitrate-response genes as well as known components of nitrate signaling.
Discussion: Upon exposure to the triggering stimulus, primed plants exhibit a different response pattern when compared to unprimed plants. This memory response might be mediated by modulating the C/N ratio. Total N-content and molecular genetics results suggest that nitrate-signaling, rather than internal N-status would be driving this nutritional memory response. We propose a mechanism that could explain the improved phenotypes observed in primed plants.
Acknowledgments: ANID–Millennium Science Initiative Program-Millennium Institute for Integrative Biology (iBio) (ICN17_022), the Center for Genome Regulation (ICN2021_044), and ANID-FONDECYT 1220594.

47. Mitochondrial E3 ligase MUL1 prevents early cardiac remodeling induced by angiotensin II. Magda C. Díaz-V[1] (magdac@ug.uchile.cl), Valeria Garrido[1], Ximena Calle-Chalco[1,2], Angélica Ortega-Muñoz[1], Mayarling F Troncoso[1,3], Claudia Muñoz[1], Francisco Pino-De la Fuente[1,4], David Silva[1], Brenda Becerra-Leiva[1], Alejandra Hernández[1], Alejandra Guerrero[1], Mario Chiong[1], Sergio Lavandero[1,5]. [1] Advanced Center for Chronic Diseases (ACCDiS), Facultad de Ciencias Químicas y Farmacéuticas & Facultad de Medicina,

University of Chile, Santiago, Chile. [2] Institute of Health Sciences, University of O´Higgins, Rancagua, Chile. [3] Department of Medical Technology, University of Chile, Santiago, Chile. [4] Cardiology Division, University of Texas Southwestern Medical Center, Dallas, Texas

Background: Hypertension is a chronic increase in blood pressure that affect the structure and function of the heart, initially promoting the cardiac remodeling (cardiac hypertrophy and fibrosis) and finally affecting the myocardial function. Angiotensin II (Ang II) is an endogenous peptide that regulate the blood pressure in physiological conditions, but the chronic high levels contribute to heart damage. Previous works showed that MUL1 mediates cardiomyocyte hypertrophy induced by phenylephrine or myristate. However, until know its relevance *in vivo* remains unexplored. We hypothesize that MUL1 deletion protect the heart for the cardiac remodeling induced by Ang II.
Methods: Mice C57BL-MUL1^{em1} (MUL1-/-) and its respective littermates WT (MUL1+/+) were treated with Ang II (1.5 mg/Kg/d) or saline (control) for 14 days. Then, we measured blood pressure, myocardial function by echocardiography, cardiac hypertrophy by the heart weight/tibia length (HW/TL) ratio and cross-sectional area (CSA) by WGA staining, and the fibrosis by Picrosirius Red staining (PSR).
Results: Both, MUL1-/- and MUL1+/+ increased the blood pressure in response to Ang II, without changes in systolic heart function (%LVEF). However, the mice MUL1-/- prevented increase HW/TL and cardiomyocyte CSA3 triggered by Ang II and the increase in % PSR area.
Conclusions: These results suggest that MUL1 play a role in the cardiac remodeling independent of the high blood pressure and in early states before heart failure development.
Funding: FONDECYT 3210443 (VG), 1240443 (SL), FONDAP 1523A0008 (SL).

48. Enzymes before the origins of life: prebiotic amyloids are catalytically active and can endure prebiotically plausible conditions. Eva Duran-Meza[1,2] (eva.lisadm@gmail.com), Claudio Castillo-Caceres[2], Octavio Monasterio Opazo[1], Rodrigo Diaz-Espinoza[2]. [1]Departamento de Biología, Facultad de Ciencias, Universidad de Chile. [2] Departamento de Biología, Facultad de Química y Biología Universidad de Santiago de Chile.

Introduction: A diverse array of macromolecules has been proposed as prebiotic candidates for their role in the chemistry preceding living organisms. Ribonucleotides can encode both hereditary and catalytic characteristics but are highly unstable, difficult to polymerize and need large sizes for function. Prebiotic routes for synthesis of peptides are diverse and more feasible but the products are still unstable and poorly functional. Recently, the assembly of peptides into highly stable intermolecular assemblies such as amyloids has emerged as an alternative scenario. Once formed, the amyloids remained functionally active even after treatment under harsh conditions. Here, we show that peptides composed of

prebiotic amino acids and with their N- and C-terminals free can spontaneously self-assemble into active supramolecular scaffolds.
Materials and Methods: Aggregation time-course experiments of the prebiotic peptides were followed by fluorescence of the amyloid-specific thioflavin-T (Th-T) probe. The ultra-structure of these prebiotic amyloids was analyzed by transmission electron microscope (TEM). For the catalytic activity, we performed a high-throughput activity assay using an adapted green malachite-based method to measure the hydrolysis of the phosphoanhydride bonds of adenosine triphosphate (ATP) and polyphosphate.
Results: Fluorescence and transmission electron microscopy studies showed that these assemblies are amyloids. Their formation can be induced by diverse experimental conditions of prebiotic relevance including different pH extremes and high salt concentrations. Moreover, these prebiotic amyloids exhibited enzyme-like behavior, catalyzing the hydrolysis of biologically relevant molecules such as ATP and polyphosphate.
Discussion: These results demonstrate that peptides with prebiotic composition and with their N- and C-terminals free can form catalytically active amyloids that can withstand prebiotically plausible conditions. Therefore, self-assembly into amyloids allows small prebiotic peptides to reach complex conformations that can act as minimalist enzyme-like catalysts, bypassing the need for large and specific sequences to achieve catalytic function before the origins of life.
Acknowledgements: FONDECYT 1211821.

49. Enzymatic cleavage for locating and inhibiting precise sequence elements (ECLIPSE) using the CRISPR-Cas12a system in *Pygoscelis adeliae* (Antarctic penguin), *Haliclona scotti* and *Geotria australis (Lamprey).* Benjamín Durán-Vinet[1] (benjamin.duran-vinet@postgrad.otago.ac.nz), Allison Miller[1], Jo-Ann Stanton[1], Sara Ferreira[1], Gert-Jan Jeunen[2], Neil Gemmell[1]. [1]Department of Anatomy, School of Biomedical Sciences, University of Otago, Dunedin, 9016, New Zealand. [2]Department of Marine Sciences, University of Otago, Dunedin, 9054, New Zealand.

Introduction: Next-generation sequencing (NGS) technologies have provided invaluable insights into genome architectures and diagnostics. However, a significant challenge in using NGS for diagnostic, gut, and environmental studies is the low ratio of target DNAs to unwanted DNA. Adaptive sequencing addresses this issue with real-time sequencing to select specific DNA sequences, but it is difficult when metabarcoding approaches use highly conserved regions such as *Cytochrome c oxidase subunit I* with Leray metazoan primers. These challenges can lead to over-represented, uninformative DNA and a truncated taxonomic profile, undermining data biodiversity. Thus, there is a need for a versatile, cost-effective tool to effectively deplete unwanted DNA, enhancing data biodiversity from sequencing.
Materials and Methods: Clustered Regularly Interspaced Short Palindromic Repeats and associated CRISPR proteins (CRISPR-Cas) have emerged as a novel, highly precise, and

programmable DNA nucleases mediated by CRISPR RNAs (crRNAs). In this study, we harness DNA-targeting LbaCas12a (Lachnospiraceae bacterium ND2006) to deplete 0.1 ng/uL of decoy plasmids containing the Leray metabarcoding amplicons from different host species, including *Pygoscelis adeliae*, *Haliclona scotti*, and *Geotria australis*. We designed two crRNAs for each species, targeting different sites of interest (i.e., primer binding regions and/or mid-amplicon regions) to study and quantify the system efficacy in depleting target DNA for future use on feces samples.
Results: Our *in vitro* results obtained via quantitative PCR (qPCR) indicate that the decoy plasmids signal was decreased by >99.5% after only 2 hours of incubation with LbaCas12a, corresponding to an average over 900-fold reduction in decoy DNA signal when using a single crRNA with a Cas12:crRNA:DNA ratio of 20:40:1. Furthermore, the use of combined crRNAs provided a doubled fold reduction (2000-fold; >99.9%). This proof-of-concept study demonstrates that LbaCas12a is a promising, cost-effective, and programmable depletion technology, termed **E**nzymatic **C**leavage for **L**ocating and **I**nhibiting **P**recise **S**equence **E**lements (ECLIPSE).
Acknowledgement: A New Zealand Royal Society Te Aparangi ¯ Marsden Fast-Start (MFPUOO2116), a University of Otago Research Grant, the Ministry of Business, Innovation, and Employment Antarctic Science Platform (MBIE ANTA1801), and the Ministry of Business, Innovation, and Employment: A toolbox to underpin and enable tomorrow's marine biosecurity system (MBIE CAWX1904) funded the cost for this project.

50. Implementation of a complementary monitoring tool for high-level disinfection (HLD) of endoscopic devices. Troy Ejsmentewicz[1] (troy.ejsmentewicz@falp.org), Gerthy Rios-Rioseco[2], Francisca Asis[2], Karen Saldivia[2], Celia Podesta-Medina[3] and Franz Villarroel-Espíndola[1]. [1]Translational Medicine unit, [2]Sterilization unit, [3]Endoscopy unit, Fundación Arturo López Pérez (FALP), Santiago, Chile.

Introduction: Gastrointestinal endoscopy is effective and safe medical procedure, and most of the endoscopes are reprocessed during the clinical practice. Guidelines for reprocessing endoscopes are focused on eliminating live bacteria, not considering residual membranes, nucleic acids, proteins, and polysaccharides as potential sources of non-specific inflammatory reactions. The objective of this work was to develop a biological tool to evaluate the performance of High-Level Disinfection (HLD) protocols.
Materials and Methods: Washing liquid from 36 endoscopes was collected before and after applying an HLD protocol. An ATP bioluminescence assay was used as routine cleaning test. The in-house monitoring assay was based on cultured peripheral blood mononuclear cells (PBMCs) exposed to washing liquid for 3 hours, and subsequent IL-1β mRNA level detection by RT-qPCR. Sensitivity was assessed using a standardized and a non-standardized HLD protocol.
Results: IL-1β mRNA was measured at three points of reprocessing: before washing (baseline), after manual washing and after an automatized reprocessing machine. A manual

washing (p=0,0014) and the reprocessing machine (p=0.0009) were equally efficient to remove pro-inflammatory agents compared to the baseline. A standardized protocol showed to be statistically better than a non-standardized one (p-value=0,0052). Non-compliance showed a significant increase the expression of IL-1β by 4-fold after manual washing and 2-fold after the reprocessing machine (p>0.001 in all cases).
Discussion: We have developed an *in-house* method based on a biological response using live cells to evaluate the efficiency of HLD protocols used for reprocessing endoscopes, and it may be implemented as part of clinical routine in our center.
Acknowledgment: FALP-LMT-2023 & 2024; ANID-FONDECYT 1221415.

51. Astaxanthin as Treatment to Ameliorates Right Ventricular Hypertrophy through Antioxidants properties in rats exposed to Chronic Intermittent Hypobaric Hypoxia.
Samia El Alam (selalam@unap.cl), Eduardo Pena, Constanza Gonzalez, Diego Aguilera. High Altitude Medicine Research Center (CEIMA), Arturo Prat University, Iquique, Chile, 1100000.

Introduction: In Chile, there is a particular exposure to high altitude termed chronic intermittent hypobaric hypoxia (CIHH), due to work shift system of days exposed at high altitude and days of rest at sea level in the long-term. CIHH can induce the development of pathologies such as high-altitude pulmonary hypertension (HAPH), where the principal consequence is the development of right ventricular hypertrophy (RVH), which can cause heart failure and eventually death. Studies have shown the detrimental effects of oxidative stress and inflammation on the development of cardiac hypertrophy. The aim of this study was to determine if astaxanthin administration treatment mitigates RVH in rats under CIHH and the association to oxidative stress.
Materials and methods: 32 male wistar rats were randomly assigned to one of the following groups (n=8): normoxia with vehicle (NX), normoxia with astaxanthin (NX+AS), chronic intermittent hypobaric hypoxia with vehicle (CIHH), and chronic intermittent hypobaric hypoxia with astaxanthin (CIHH+AS). CIHH was simulated with hypobaric chamber (4.600 m) for 2 days and 2 days at sea level, for 30 days. Astaxanthin was administered once daily (50 mg/kg body weight). RVH, Nox2, lipid peroxidation, antioxidants (CAT, GPx) and 8-isoprostane were measured.
Results: Astaxanthin administration decrease the development of RVH and the Nox2 expression and lipid peroxidation in rats exposed to CIHH, however, the GPx and CAT activity in RV and urine 8-isoprostane did not showed differences in the groups of study.
Discussion: These results indicate an oxidative stress status after CIHH exposure where astaxanthin administration could attenuates this condition. Moreover, RVH might be associated with oxidative stress, however more studies are needed.
Acknowledgements: FONDECYT INICIACION 11230214.

52. Lack of Neuraminidase 1 provides protection against Experimental Autoimmune Encephalomyelitis. Emilia Escalona[1,2] (emilia.escalona@uautonoma.cl), Sofía Albornoz-Muñoz[1], Enzo Bonacic-Doric[1], Celia Salazar[1], Andrés Herrada[1] and Noelia Escobedo[1]. [1]Lymphatic Vasculature and Inflammation Research Laboratory, Instituto de Ciencias Biomédicas, Facultad de Ciencias de la Salud, Universidad Autónoma de Chile, Talca, Chile. [2]Multidiciplinary Agroindustry Research Laboratory, Instituto de Ciencias Biomédicas, Facultad de Ciencias de la Salud, Universidad Autónoma de Chile, Talca, Chile.

Introduction: Multiple sclerosis (MS) is a neurodegenerative and autoimmune disease affecting the central nervous system (CNS), characterized by inflammation and demyelination, in which microglia and CNS-infiltrating macrophages can have either pro-inflammatory (M1) or repairing (M2) roles. Neuraminidase 1 (Neu1) is a sialidase involved in the human neurodegenerative disorder sialidosis and modulates immune functions. However, its impact on macrophage polarization and in autoimmune disorders affecting the CNS, such as MS, has not yet been reported.
The aim of this work was to determine the effect of Neu1 absence on M1/M2 macrophage polarization and MS pathogenesis in murine models.
Materials and Methods: Clinical manifestations, CNS-immune cell infiltration, and M1/M2 microglia/CNS-infiltrating macrophage polarization were evaluated in *WT* and *Neu1*-knockout *(Neu1$^{-/-}$)* mice with experimental autoimmune encephalomyelitis (EAE) using EAE score and flow cytometry. Additionally, Neu1 effect on M1/M2 macrophage polarization was evaluated *in vitro* using *WT* and *Neu1*-deficient bone marrow-derived macrophages (BMDMs).
Results: *Neu1$^{-/-}$* mice did not exhibit clinical manifestations of EAE compared to *WT* mice. Immune cell infiltration analysis revealed that *Neu1$^{-/-}$* mice showed significantly less immune cell infiltration and had fewer microglia/CNS-infiltrating macrophages in their CNS. Additionally, *Neu1*-deficient macrophages exhibited a significantly less inflammatory phenotype both *in vitro* and *in vivo.*
Discussion: Together, these experiments demonstrate that Neu1 plays an essential role in EAE pathogenesis, macrophage polarization, and CNS-immune cell infiltration in this autoimmune disease.
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53.Molecular characterization of TET2 on metabolic reprogramming and microglial inflammatory response. Liliana Espíndola [1,2], Angélica Ríos[2], Cheril Tapia[3], Alejandra Hernández[1], Mayarling F Troncoso[1], Claudia Muñoz[1], Erik López-Gallardo[1], Marcelo Kogan[1], Nibaldo Inestrosa[2], Sergio Lavandero[1,4]. [1]Advanced Center for Chronic Diseases (ACCDiS), Faculty Chemical & Pharmaceutical Sciences & Faculty of Medicine, Universidad de Chile; [2]Centre of Excellence in Biomedicine of Magallanes, CEBIMA, Universidad de

Magallanes. [3]Faculty of Medicine & Science, Universidad San Sebastián; [4]Cardiology Division, UT Southwestern Medical Center, Dallas, Texas, USA.

Introduction: Alzheimer's disease (AD) is characterised by an impairment in memory and higher cortical functions. Microglia, commonly known as the macrophages of the central nervous system, are essential for proper neuronal function and require the transport of glucose and glucose-derived metabolites to perform their inflammatory response functions. *TET2* (Ten-Eleven Translocation 2), an enzyme involved in DNA demethylation, is an important regulator of AD-related microglial neuroinflammation. *TET2* may induce metabolic reprogramming in response to an inflammatory stimulus. We hypothesize that down-regulation of TET2 decreases inflammation through metabolic reprogramming of the glycolytic pathway in □-amyloid-stimulated microglia cells.
Methods: We performed the transfection of BV-2 microglial cell line using Lipofectamine 3000 and evaluated the differences in the level of expression of the *TET2* gene. Additionally, we examined gene expression related to the inflammatory response and the quantification of energy consumption by Seahorse XFe 96 analyzer, WB, IF, and RT-qPCR. These evaluations were conducted on microglia BV2 cell lines treated with LPS, amyloid conditioned media (referred to as 7PA2-CM) and □-amyloid aggregated samples.
Results: Our data showed that the administration of LPS and 7PA2-CM at varying concentrations resulted in an enhancement of the expression of proinflammatory markers such as IL-1, IL-6, inducible nitric oxide synthase (iNOS), TNF□ and p-NFkB levels in BV2 cells. Interestingly, elevated levels of RhoA were observed under the same experimental conditions. A significant increase in the expression of GLUT1 and HKII was observed when treated with LPS and 7PA2-CM at a 1:20 dilution. Regarding the microglia marker, Iba-1, it showed much higher levels when treated with 7PA2-CM at a 1:5 dilution.
Conclusion These preliminary results highlight the relationship between metabolic reprogramming and inflammation in microglia.
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54. NUAK1 role in the regulation of the nuclear PFKP/YAP/TAZ axis in breast and colorectal cancer. Luis Espinoza-Francine (Luespinoza2018@udec.cl); Viviana Coliboro-Dannich; Alejandro Farías; Pablo Parra; Roxana Pincheira; Ariel Castro. Signal Transduction and Cancer Laboratory, Department of Biochemistry and Molecular Biology, Faculty of Biological Sciences, University of Concepción, Concepción, Chile.

Introduction: NUAK1 is a ser/threo kinase associated with bad prognosis in several cancer types, including breast (BC) and colorectal cancer (CRC). However, mechanistic insights into how NUAK1 contributes to cancer are still scarce. We previously found that NUAK1 interacts with PFKP, the platelet isoform of the glycolytic enzyme phosphofructokinase-1 (PFK1), which is also overexpressed and associated with poor prognosis in several cancer types. The canonical function of PFKP is the regulation of glycolytic flux. PFKP regulates

glycolysis in the cytosol; however, it also localizes in the nucleus of some cell types. Regarding nuclear functions, PFKP promotes tumorigenesis via the interaction and stability of transcription factors (TFs), such as YAP/TAZ/TEAD1 and c-Myc. Because previous studies indicated that NUAK1 could also regulate the YAP/TAZ/TEAD1 axis, we evaluated whether NUAK1 promotes PFKP nuclear translocation and impacts YAP/TAZ/TEAD1-driven gene expression in BC and CRC cells.
Material and Methods: MDA-MB-231 (BC), MCF-7 (BC), and HCT-116 (CRC) cells were treated with the NUAK1-specific inhibitor HTH-01-015 to evaluate nuclear levels of PFKP, TEAD1, YAP, and TAZ by Western Blot. RT-qPCR measured gene targets, and a luciferase reporter assay evaluated TEAD1 activity.
Results: We performed subcellular fractionation and demonstrated that PFKP is present in the nucleus of BC and CRC cells. PFKP nuclear localization was β-importin-dependent, demonstrated by a coimmunoprecipitation assay and importazole treatment. NUAK1 promoted PFKP nuclear localization and increased YAP nuclear levels. Accordingly, NUAK1 induced the mRNA expression of YAP target genes (Cyr61, CTGF, and FoxM1). Additionally, NUAK1 also regulated TEAD1-dependent transcriptional activity.
Discussion: Our results suggest a role for NUAK1 in promoting PFKP nuclear localization and regulation of gene expression in BC and CRC cells.
Acknowledgment: FONDECYT Regular: 1201215 and 1241771.

55. An optogenetics-based approach highlights the role of a horizontally acquired region in yeast fermentation under low nitrogen availability. David Figueroa[1,2] (david.figueroa@alumnos.uach.cl), Diego Ruiz[1,2], Nicolò Tellini[3], Matteo De Chiara[3], Eduardo Kessi-Pérez[4,5], Claudio Martínez[4,5], Gianni Liti[3], Amparo Querol[6], José Manuel Guillamón[6], Francisco Salinas[1,2]. [1]Laboratorio de Genómica Funcional, Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile. [2]ANID – Millennium Science Initiative – Millennium Institute for Integrative Biology (iBio), Santiago, Chile. [3]Université Côte d'Azur. CNRS, INSERM, IRCAN, Nice, France [4]Centro de Estudios en Ciencia y Tecnología de los Alimentos (CECTA), Universidad de Santiago de Chile (USACH), Santiago, Chile. [5]Departamento de Ciencia y Tecnología de los Alimentos, Facultad Tecnológica, Universidad de Santiago de Chile (USACH), Santiago, Chile. [6]Departamento de Biotecnología de los Alimentos, Instituto de Agroquímica y Tecnología de los Alimentos (IATA-CSIC), Valencia, Spain.

Nitrogen limitation in grape must is the primary cause of stuck fermentations during winemaking. In *Saccharomyces cerevisiae*, a genetic segment known as region A, harboring 12 protein-coding genes, was horizontally acquired from a phylogenetically distant yeast species. This region, predominantly found in wine yeast genomes, contains genes linked to nitrogen utilization. Despite its potential significance, the contribution of region A to the fermentation process remains largely unexplored. In our study, we used a wine yeast strain to investigate the role of region A in fermentation. First, we sequenced the genome of the

wine yeast strain using long-read sequencing, confirming that the region is present as a single copy. We then employed an optogenetic system to precisely regulate the expression of each gene within this region, generating a collection of 12 strains for light-activated gene expression. To evaluate the role of these genes during fermentation, we assayed microculture and fermentation experiments in synthetic must with varying nitrogen availability. Our results indicate that altering gene expression within region A can significantly affect growth parameters and fermentation rates. Furthermore, we demonstrated that the expression of certain genes in region A is crucial for completing the fermentation process and preventing stuck fermentations under low nitrogen conditions. Overall, our optogenetics-based approach highlights the role of region A in yeast fermentation under nitrogen-limited conditions.

56. Role of the Primary cilium in maintaining the contractile phenotype of vascular smooth muscle cells. Flores-Vergara R[1,2,3], Larraín-Segura R[1,2,3], Abarca V[1,2], Mancilla G[1,2,5], Verdejo H[2,5], Chiong M[2,3,5*], Pedrozo Z[1,2,3*]. [1]ICBM, Faculty of Medicine, Universidad de Chile. Chile. [2]Advanced Center for Chronic Diseases (ACCDiS), Faculty of Chemical and Pharmaceutical Sciences & Faculty of Medicine, Universidad de Chile. Chile. [3]CEMC, Faculty of Medicine, Universidad de Chile. Chile. [4]Department of Pharmacological & Toxicological Chemistry, Faculty of Chemical & Pharmaceutical Sciences, Universidad de Chile. Chile. [5]Cardiovascular Signaling Laboratory, Division of Cardiovascular Diseases, Faculty of Medicine, Pontificia Universidad Católica de Chile. Chile.

Introduction: Vascular smooth muscle cells (VSMCs) regulate blood pressure through their contractile proteins. Under pathological conditions, VSMCs switch from a contractile to a secretory phenotype, contributing to the development of vascular diseases such as hypertension. The primary cilium is a cellular organelle specialized in the transduction of chemical and mechanical signals. Loss of the primary cilium induces ciliopathies, which predispose individuals to obesity and renal and vascular diseases. However, the role of this organelle in maintaining the contractile phenotype of VSMCs remains unknown.
Objective: To study the role of the primary cilium in maintaining the contractile phenotype of vascular smooth muscle cells *in vitro* and *in vivo*.
Methodology: The A7r5 cell line (VSMC) was used. Primary cilia were disassembly using a siRNA-IFT88. The phenotype was assessed by Western-blot markers (α-SMA, SM22-α, Calponin). Migration was assessed by wound healing assay. The primary cilium was identified by immunofluorescence (Acetylated Tubulin). Thoracic aortas from eight-month-old spontaneously hypertensive rats (SHR) and their normotensive control Wistar-Kyoto rats, (WKR) were used to determine the percentage of ciliated cells and their contractile protein content. Statistical analysis was performed by t-test or ANOVA, followed by Tukey test. Differences were significant when $p < 0.05$. n=3-5.
Results: Our results show that, disassembly of primary cilia in vascular smooth muscle cells reduces contractile markers and increases cell migration. In a spontaneously hypertensive

animal model, a lower percentage of ciliated cells is observed, together with a decrease in contractile proteins.
Conclusions: The primary cilium is crucial in maintaining the contractile phenotype of vascular smooth muscle cells.
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57. Obtention and analysis of the microbial biopolymer poly (γ) glutamic acid and elucidation of its potential benefits for agricultural crops. Puopolo A[1], Loser U[1], Bianucci E[2], Furlan AL[1] (afurlan@exa.unrc.edu.ar). [1]Instituto de Investigaciones Agrobiotecnológicas (INIAB, CONICET-UNRC), Río Cuarto, Córdoba. [2] Laboratorio de Fisiología Vegetal, Facultad de Biociencia, Universitat Autònoma de Barcelona, E-08193, Bellaterra, España.

Introduction: The positive effect of a glutamic acid polymer called poly-γ-glutamic acid (γ-PGA) produced by plant growth-promoting bacteria (PGPB) is suggested as a biotechnological tool to improve crops of agricultural interest. The cultivation of peanuts (*Arachis hypogaea* L.) is of great economic importance in the province of Córdoba (Argentina), the main producer of the legume at a national level. Thus, the objective of this study was to obtain and analyze the microbial biopolymer γ-PGA and to elucidate its potential benefits for agricultural crop.
Materials and methods: Rhizospheric soil of peanut plants was diluted aseptically, grew in LB medium and bright mucous colonies were selected. Broth supernatants were precipitated with ethanol in saline cold solution. The pellet was solubilized in water and plated on agarose or polyacrylamide gels and compared with a commercial γ-PGA standard by staining with methylene blue. Next, the γ-PGA was analyzed in H^1-NMR and HPLC-MS-MS. The isolates were identified by amplification of the 16s ribosomal gene from total genomic DNA and NCBI BLASTN. Then, its effect on peanut growth was evaluated in controlled conditions.
Results: Rhizospheric bacteria from genera *Bacillus* (strains RF21; B3B; B61 and RF64) and *Peribacillus* (strain RF11) isolated from peanut plants produced a homopolymer of glutamic acid, namely γ-PGA. The strains differed in the length of the polymer produced. The inoculation of native bacteria producing long γ-PGA polymers had a positive effect on peanut growth, as revealed by increases in biomass and nodulation.
Discussion: The microbial biopolymer γ-PGA produced by rhizospheric bacteria from genera *Bacillus* and *Peribacillus* has a potential use as a bioinput. Future studies will help to understand its role in the metabolism of the plant-microorganism interaction and the physiology of biological nitrogen fixation.
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58. Role of microRNAs induced by knockdown of mitochondrial non-coding antisense RNAs in the DNA damage response in MDA-MB-231 breast tumor cells. Constanza Gabilán (c.gabilanaraya@gmail.com), Nicole Farfán, Verónica A. Burzio. Department of Biological Sciences, Faculty of Life Sciences; Institute of Biomedical Sciences, Faculty of Medicine, Universidad Andrés Bello, Santiago.

Introduction: Triple-negative breast cancer (TNBC) is the most aggressive and treatment-refractory form of breast cancer, with the lowest survival rate of all subtypes. Chemotherapy with DNA-damaging drugs is the first line of therapy; however, these drugs also cause damage to healthy cells, affecting patients' quality of life. Therefore, more selective and effective therapies are needed. Knockdown (KD) of mitochondrial non-coding antisense RNAs (ASncmtRNAs) with antisense oligonucleotide (ASO) 1537S induces apoptotic death of MDA-MB-231 TNBC cells, accompanied by induction of DNA damage, which is likely mediated by the observed reduction in levels of proteins involved in genomic integrity maintenance (Aurora Kinase A and Topoisomerase IIa). Concomittantly, the treatment also induces an increase in the levels of microRNAs miR-1973 and miR-4485-3p, whose sequences are contained within ASncmtRNA-2. This study aims to determine if the DNA damage elicited by ASncmtRNA KD in tumor cells is mediated by miRNAs miR-1973 and miR-4485-3p MDA-MB-231 cells treated with ASO-1537.
Materials and Methods: ASOs 1537 and control (ASO-C), and inhibitors of miR-1973 and miR-4485-3p (and a control inhibitor) were co-transfected into MDA-MB-231 cells using LipofectAmine2000 for 48 h. DNA damage was observed using the comet assay and induction of the DNA damage response (DDR) was determined by Western blot quantification of the DDR marker γ-H2AX.
Results: A decrease in DNA damage was observed in ASO-1537-treated cells co-transfected with miR-1973 and miR-4485-3p inhibitors, compared to the control inhibitor.
Discussion: Our results suggest that miR-1973 and miR-4485-3p mediate the DNA damage observed after KD of ASncmtRNA in MDA-MB-231 cells.
Acknowledgment: FONDECYT 1230760.

59. Synergetic effect of native phosphate solubilizing bacteria in the bioleaching of rare earths from tailings. Gabriel Gálvez [1,2] (gabriel.galvez@uoh.cl), Jaime Ortega [1,2,] Mauricio Latorre [1,2,3]. [1]Laboratorio de Bioingeniería; Instituto de ciencias de la ingeniería; Universidad de O'Higgins, Rancagua, Chile. [2]Centro de biología de sistemas para el estudio de comunidades extremófilas de relaves mineros (SYSTEMIX), Universidad de O'Higgins, Rancagua, Chile. [3]Laboratorio de bioinformática y expresión génica, INTA, Universidad de Chile, Santiago, Chile.

Introduction: Rare earths elements are key elements in the generation of clean energy, which are increasingly being used. The Cauquenes tailings, in Chile has a high concentration of REEs, this concentration, together with the existence of bioleaching

bacteria, as well as the combined use of bacteria to enhance bioleaching, open a new field within mining. The present work studies bioleaching of native bacteria from the Cauquenes tailings and the capacities of synergy.
Materials and Methods: Samples from Cauquenes tailings were used to isolate bacteria, identified via Gram staining and 16S sequencing. Phosphate solubilization tests in PVK medium identified effective bioleaching bacteria. Selected isolates, showing larger halos in the PVK medium than *Klebsiella aerogenes*, were tested for synergistic effects to enhance solubilization.
Results: A total of 60 isolates were identified, 11 isolates could solubilize phosphate. Isolates 1 and 2, were selected for their high potential and tested for synergy. Groups 1 (Isolate 1) and 2 (Isolate 2) increased phosphate solubilization compared to isolates. Both could bioleach REEs from Cauquenes tailings, with isolates solubilizing over 5 times more than *Klebsiella aerogenes*, and Groups solubilizing 40% more than isolates.
Discussion: We hope that these results will allow us to propose a biotechnological option for obtaining REEs from tailings.
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60. Nudt3 endo-polyphosphatase activity and its role in amyotrophic lateral sclerosis progression. Polett Garcés (p.garcsgimnez@uandresbello.edu), Constanza Moraga, Kevin Leiva, Brigitte van Zundert, Martín Montecino. Instituto de Ciencias Biomédicas (ICB), Facultad de Medicina, Universidad Andrés Bello, Santiago, Chile.

Introduction: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the loss of motoneurons in the brain and spinal cord. We recently discovered that excessive release of inorganic polyphosphate (polyP), a ubiquitous negatively charged biopolymer, by ALS astrocytes kills motoneurons. Our research addresses the role in ALS of Nudt3, a mammalian endo-polyphosphatase that degrades polyP in the presence of Zn^{2+}. We hypothesize that differential expression of Nudt3 in wild-type and ALS astrocytes alters polyP levels and motoneuron toxicity.
Methodology: We assessed the activity of human recombinant Nudt3 with synthetic polyP of various lengths in the presence and absence of Zn^{2+}. Additionally, we determined Nudt3 protein and transcript levels in the spinal cord of asymptomatic and diseased ALS transgenic mice carrying SOD1 mutations (mutSOD1). We also analyzed postmortem samples of the cerebral cortex and spinal cord from patients with ALS. Furthermore, we established a polyP-zymogram assay seeking to identify novel polyP-regulating enzymes (polyphosphatases and polyP kinases) in primary astrocytes.
Results: Nudt3-Zn^{2+} exhibits endo-polyphosphatase activity on synthetic long-chain polyP (130 and 700 Pi). Nudt3 mRNA expression is decreased in the spinal cord of symptomatic

mutSOD1 mice, whereas Nudt3 protein is reduced in astrocytes at early asymptomatic stages. Comparable results are found when tissues from ALS patients versus healthy individuals are analyzed. Importantly, zymogram migration bands from WT primary astrocyte-derived samples analyzed by mass spectrometry, revealed an enrichment of enzymes with unrelated kinase activity in bands reflecting proteins interacting with polyP.
Conclusions and perspectives: Nudt3-Zn^{2+} degrades long-chain polyP, comparable to that reported in brain cells. Reduced Nudt3 expression in the spinal cord of symptomatic mutSOD1 mice correlates with high polyP levels in astrocytes, consistent with previous findings in postmortem ALS patient samples. Together, these results support the need of further evaluating the role that Nudt3 and newly discovered enzymes play as potential regulators of polyP levels in control and ALS astrocytes.
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61. Role of FaMYC2 as a transcriptional regulator of the gene encoding ascorbate peroxidase (FaAPX1) in strawberry (*Fragaria* x *ananassa*). Vanessa Gonzalez-Garrido[1,2] (vgonzalezg@outlook.es) & Carlos R. Figueroa[1, 2]. [1]Laboratory of Plant Molecular Physiology, Institute of Biological Sciences, Universidad de Talca, Chile. [2]Millenium Nucleus for the Development of Super Adaptable Plants (MN-SAP), Santiago, Chile
The commercial strawberry (*Fragaria* x *ananassa*) stands out for its high levels of ascorbic acid, a key molecule within the ascorbate-glutathione cycle in plant cells, responsible for the detoxification of hydrogen peroxide (H_2O_2) through the action of the ascorbate peroxidase (APX) enzyme. In strawberry, the application of methyl jasmonate (MeJA) caused an increase in APX activity, suggesting that the jasmonate (JA) signaling pathway is acting through its pivotal transcription factor (TF) MYC2. Thus, in the present investigation it was proposed to characterize FaMYC2 as a potential TF for *FaAPX1* gene. Coding sequences for APX1 were identified in the *F.* x *ananassa* 'Camarosa' genome, mapping the frequency and distribution of MYC2-binding cis-elements (G-boxes) given the ability of this TF to interact as a dimer or tetramer. Transactivation capacity was studied in vitro using a yeast one-hybrid (Y1H) assay on promoter regions of four allelic copies of *FaAPX1*. Isolation of promoter regions revealed that the *FaAPX1-3* promoter had a fragment 200 to 300 bp smaller than expected, indicating a possible deletion or error in genome annotation. Protein-DNA interaction supported in silico predictions showing the binding capacity of MYC2 on the isolated sequences, pointing to this TF as a potential regulator of *FaAPX1* transcription. The promoters of the *FaAPX1-2* and *FaAPX1-4* copies stood out for their transactivation capacity by FaMYC2 in a selective medium attributing this to the high number of G-boxes and the consequent high probability of tetramer formation. This fact suggests that the frequency and distribution of *cis* elements influence the recognition capacity of MYC2. Further research is needed to understand the nature and strength of this specific interaction between the various

FaAPX1 copies by *in vivo* experimental approaches, as well as to quantitatively link the abundance of *cis* elements and their relationship with MYC2 in response to JA.
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62. Search for Structural Determinants of osmotic Permeability in Aquaporins. González-Aja Benjamín (begonzalez2020@udec.cl), Martínez-Oyanedel José. Laboratorio de Biofísica Molecular, Departamento de Bioquímica y Biología Molecular, Facultad Ciencias Biológicas, Universidad de Concepción.

Water scarcity is a critical global issue. In 2021, 2 billion people lived in water scarcity, and in 2022, 1.7 billion people consumed water from contaminated sources. Anthropogenic and environmental factors increase the percentage of the population without access to potable water (28% in 2022). We must optimize water purification methods. Reverse osmosis is a water purification process that needs optimization due to its requirement for higher pressure for filtration and issues with membrane fouling and deterioration. One solution is aquaporin (AQP)-based membranes. AQPs are transmembrane proteins that facilitate the transport of water, small neutral solutes, and gases. This project focuses on in silico optimization of AQP permeability, aiming to design an AQP that enhances osmotic water permeability.
From 256 structures available in the PDB, representatives were selected based on resolution, clash score, and Rwork/Rfree factor. Structural alignments (PyMOL) and multiple sequence alignments (SeaView) were performed, and a guide tree (Mega) was created, consisting of 4 clades and 7 representative proteins. These were characterized by the amino acids forming the pore (MOLE) and their contribution to pore stability using alanine scanning (Foldx). The structural determinants of permeability were sought for observation. Finally, molecular dynamics were used to evaluate osmotic water permeability (GROMACS), and based on these results, specific mutations were proposed to increase AQP permeability.
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63. Outer membrane vesicles from *Helicobacter pylori* (*Hp*) promote NF□B activation mediated by TLR2 in astrocytes. María Fernanda González C.[1,2] (mfe.gonzalez@gmail.com), Andrew F. G. Quest[1,2] and Lisette Leyton[1,2]. [1]Cellular Communication Laboratory, Faculty of Medicine, University of Chile. 2Advanced Center for Chronic Diseases (ACCDiS), Faculty of Medicine, University of Chile.

Introduction: *Helicobacter pylori (Hp)* infects the stomach of 70% of the Chilean population and presence of the bacterium is connected to gastric diseases and has recently also been associated with extra-gastric pathologies, including neurodegenerative diseases. The mechanisms by which *Hp* is related to extra-gastric diseases have been linked to the release

of nanovesicles from *Hp*, referred to as Outer Membrane Vesicles (*Hp*-OMVs). In a mouse model, these *Hp*-OMVs enter the circulatory system and reach the brain, where they promote astrocyte reactivity and NF-κB activation. However, the specific mechanisms by which *Hp*-OMVs exert these effects are unknown. Available evidence suggests that Toll-like receptor 2 (TLR2) may be relevant.
Materials and Methods: we isolated and characterized *Hp*-OMVs by Nano Tracking Analysis (NTA) and Immunoblotting. Astrocytes were then incubated with *Hp*-OMVs, with or without a blocking antibody against TLR2 (aTLR2), and NF-κB activation was evaluated by immunoblotting and immunofluorescence.
Results: The size of the *Hp*-OMVs ranged from 100-150 nm (mean 128.3 +/- 14.4) and vesicles were positive for the virulence factors CagA, VacA and urease. The treatment of astrocytes with *Hp*-OMVs increased the phosphorylation of (pS536p65) NF-κB (two-fold) and NF□B (p65) translocation to the nucleus (six-fold). Furthermore, when astrocytes were pre-treated with a-TLR2, both NF-κB phosphorylation and NF-κB nuclear translocation were significantly reduced.
Discussion: Taken together, the results indicate that *Hp*-OMVs may promote astrocyte reactivity through interaction with TLR2. Ongoing experiments will identify the virulence factor responsible for this effect.
Acknowledgment: FONDECYT Post-doctoral grant N□ 3230227 (MFG); CONICYT-FONDAP [15130011], FONDAP Continuation project [1523A0008] and ANID-FONDECYT-Regular [1210644] (AFGQ), [1200836, 1240888] (LL).

64. Unraveling the role of DcHY5 in photomorphogenesis and carotenoid synthesis.
D`Alencon, Triana, González-Calquín, Christian; Fuentes, Juaquín; and Stange, Claudia. Plant Molecular Biology Centre, Department of Biology, Faculty of Sciences, Universidad de Chile, Las Palmeras 3425, Ñuñoa, Santiago, Chile

Introduction: HY5 is a bZIP transcription factor described as a positive regulator of photomorphogenesis and carotenoid synthesis in plants. In *Arabidopsis thaliana*, AtHY5 is activated by photoreceptors such as AtPHYA to induce the expression of carotenoid biosynthetic genes, like *AtPSY*. *Daucus carota* (carrot) accumulates high levels of carotenoids in its storage root when it grows underground, and PHYA is required for this process.
Methods: By means of bioinformatics, subcellular localization, relative gene expression and an *in vivo* transient expression assay we present evidence of DcHY5 functionality to unravel the mechanism of carotenoid synthesis in carrot storage root.
Results: We determined that DcHY5 has a 75% sequence identity with AtHY5 and a highly conserved bZIP domain, it localizes to the nucleus and interacts with DcPHYA. The study of its promoter shows responsive elements to light, hormones, and stress, among others, whilst the regulatory network performed by STRING revealed its co-expression with factors such as COP1, BBX21, BBX25, SPA1, RUP1 and RUP2. At the functional level, we

determined that it promotes the expression of the *DcPSYs* and the synthesis of carotenoids. This assay was carried out by infiltrating transgenic tobacco plants that express GFP under the command of the native *DcPSY1* and *DcPSY2* promoters – serving as reporter lines– with 35sCaMV:DcHY5. We observed an increase in GFP fluorescence in contrast to plants infiltrated with empty vectors and a boost in total carotenoid content of around two-fold.
Discussion: Together, these results suggest that DcHY5 plays a positive role in the biosynthesis of carotenoids in *D. carota* and could be interacting with the promoters of *DcPSY1* and *DcPSY2*.
Funding: Fondecyt 1221399.

65. The Characterization of PrMADS10 and PrMADS11 Transcription factor from Pinus radiata. Joselin Guajardo (joguajardo@utalca.cl), Felipe Valenzuela-Riffo, Yazmina Stappung, María Alejandra Moya-León, Raúl Herrera**.** Instituto de Ciencias Biológicas, Universidad de Talca

Introduction: The loss of trunk verticality triggers a series of molecular responses that coordinate physiological and biochemical changes, allowing the recovery of straight growth. Trunk morphological changes and differential accumulation of phenolic compounds have been visualized. Changes in secondary cell wall components affect wood quality and are modulated by transcription factors (TF). MADS-boxes have been identified probably involved in lignin biosynthesis activation.
Materials and Methods: Sequences of MADS-box TFs were obtained through genome walker strategy and analyzed by bioinformatic tools. *Arabidopsis thaliana* Columbia ecotype plants were transformed using the floral dip method, and overexpressing T3 mutant lines were obtained. Accumulation of transcripts and lignin content were quantified in three lines from each mutant, and differences at metabolic level were identified.
Results: PrMADS-box TFs under study belong to MADS-box family group II sharing the MIKC structure but differ in the length of their C domain. The CDS of *PrMADS10* codifies for a protein of 194 AA, and *PrMADS11* for 165 AA. *PrMADS10* and *PrMADS11 A. thaliana* overexpressing mutants showed an increase in lignin content and displayed a marked increment in the expression of genes of the phenylpropanoid pathway driven to the biosynthesis of lignin. EMSA assays and structural bioinformatics analyses confirmed that PrMADS10 and PrMADS11 interacts with CArG-box sequences, *cis* elements described in promoter zones of cell wall genes in *P. radiata*. In addition, the data indicates that these TFs are transcriptional regulators of other TFs and different genes involved in remodeling of cell wall.
Discussion: These TFs modulate gene expression of several molecular pathways, including other TFs and genes involved in cell wall remodeling. The increment of lignin content and modulation in cell wall dynamics could be an indication of their key role in response to trunk inclination.
Acknowledgment: FONDECYT N° 1241579 and ANILLO ATE220043.

66. Towards minimalistic esterases: catalytic activity of amyloids self-assembled with small peptides. Hardy Guzmán[1] (hardy.guzman@usach.cl), Jenny M. Blamey[1,2], Rodrigo Díaz[1]. [1]Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile. [2]Fundación Científica y Cultural Biociencia, Santiago, Chile.

Introduction: Amyloids are highly ordered peptide aggregates consisting of beta-sheets motifs, which are very stable due to intermolecular hydrophobic interactions and hydrogen bonds. Despite being historically associated with diseases, they have unique physical and chemical properties that can be very relevant for biotechnological applications. The recent discovery that a variety of peptides inspired on the active site of carbonic anhydrase can self-assemble into catalytically active amyloids with esterase-like activity has paved the way to develop minimalistic enzyme-like amyloids. Hydrolytic enzymes such as esterase are highly valuable in the biotechnology industry. They are involved in several key process, including the degradation of adipose waste from various industrial sources, esterification and consequent generation of high-value products. The aim of this work is to evaluate the hydrolytic activity on ester bond of fatty acids model by amyloids self-assembled.
Materials and methods: We assayed the hydrolytic activity of catalytically active amyloids, testing a panel of seven small peptides, using p-nitrophenyl (pNP) derived esters of model fatty acids (pNP acetate, pNP butyrate, pNP valerate, pNP octanoate and pNP laurate).
Results: The activities showed enzyme-like saturation curves for most peptides that fitted well to a Michaelis-Menten model. The kinetic parameters were analyzed based on the hydrophobic nature of the lipids.
Conclusions: We expect these results to greatly improve our understanding of amyloid-based hydrolysis of industrially relevant substrates, for the future implementation of minimalistic esterases. **Acknowledgment:** ANID, Proyecto Explorador Nº13220108 and scholarship 23/04/2024 - 2543 Vicerrectoria de Postgrado from the University of Santiago de Chile.

67. MicroRNAs Recovered From Biobank Samples As Biomarker Candidates In Cancer Research. Kevin Guzmán-Nawrath[1,5,6,7] (kevin.guzman@ug.uchile.cl), Evelyn Prodan[2,3], Agustina Chimento,[2,4], Emilia Matiacich[2], Sofía Herrera[2,4], Andrew Quest[5,6], Carolina Cristina[2,3], Lorena Lobos-González[5,6,7]. [1]Magíster en Bioquímica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile. [2]Laboratorio de Neuroendocrinología/Fisiopatología de la Hipófisis; Centro de Investigaciones Básicas y Aplicadas (CIBA), Universidad Nacional del Noroeste de la Pcia. de Bs. As. Junín B6000, Argentina. [3]Centro de Investigaciones y Transferencia CIT NOBA,Consejo Nacional de Investigaciones Científicas y Técnicas (UNNOBA-UNSAdA-CONICET). [4]Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, CIC, Calle 526 e/ 10 y 11, La Plata, Buenos Aires, Argentin. [5]Centro Avanzado de Enfermedades Crónicas (ACCDiS), Independencia, Santiago, Chile. [6]Laboratorio de Comunicaciones Celulares, Instituto de

Ciencias Biomédicas (ICBM), Facultad de Medicina, Universidad de Chile. [7]Centro de Medicina Regenerativa, Facultad de Medicina, Universidad Del Desarrollo.

The diverse spectrum of existing RNAs is widely involved in various physiological and pathological processes. Numerous efforts have been made to identify suitable microRNAs that can function as biomarkers, especially in tumor biology. In clinical research, where large or different types of samples are not always available, obtaining molecular information that helps to diagnose and monitor patients is crucial. For this reason, we standardized RNA extraction protocols using TRIzol on formalin-fixed-paraffin-embedded tumors (FFPE), which are not often used for this purpose despite being widely available in biobanks, in addition to blood plasma (BP) and serum samples that are commonly used in clinical settings.
FFPE xenograft tumors from MDA-MB-231 (FFEP-MDA) or PC-3 (FFEP-PC3) cells were used, in addition to BP or serum from healthy donors, to evaluate the presence of microRNAs by Stem-Loop RT-qPCR. We detected SNORD44, a small nucleolar RNA commonly used as an endogenous control, at Cq of 21,3 for FFEP-MDA and 24,5 for FFEP-PC3. Additionally, Cqs for plasma or serum were around 30,6 for SNORD44; and, 29,3 and 32,5 for the candidate biomarkers miR-23b and miR-26a respectively.
The results show that TRIzol RNA extraction combined with the Stem-loop RT-qPCR protocol, offers the possibility of recovering large amounts of biological information trapped in paraffin-embedded tumors, as well as from commonly used blood derivatives. This approach broadens the range of material available for retrospective studies, thereby favoring translational research.
While technical challenges remain, extracting RNA from such fixed biological samples represents important advantages, especially in clinical research where often only one type of sample is available. Having sample alternatives permits more flexible and adaptable research, enabling us to answer complex questions, particularly in biomolecular diagnostics.

68. Characterization of the glycogen phosphorylase from the methanogenic archaeon *Methanococcus maripaludis*: a functional and regulatory study of a PLP-independent phosphorylase. Nicolás Herrera-Soto (Nicolas.herrera.s@ug.uchile.cl), Felipe González-Órdenes, Leslie Hernández-Cabello, Gabriel Vallejos-Baccelliere. Víctor Castro-Fernández, Victoria Guixé. Laboratorio de Bioquímica y Biología Molecular, Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

The glycogen phosphorylase (GP) enzyme degrades the glycogen polymer into glucose-1-phosphate. All glycogen phosphorylases studied employ the pyridoxal-5'-phosphate (PLP) cofactor, bound to a strictly conserved lysine residue and essential for the phosphorolytic cleavage of glycogen. However, this cofactor is not present in the GP enzyme (MmGP) from the archaeon *Methanococcus maripaludis,* even though it displays glycogen phosphorylase activity. A multiple sequence alignment revealed that the strictly conserved lysine residue

involved in PLP binding is replaced by threonine in all the GP enzymes from the order *Methanococcales*. Moreover, scarce kinetic characterization or allosteric regulation has been described for GP enzymes from archaea despite the key role of this enzyme in sugar metabolism. In this work, we characterize kinetically and spectroscopically the MmGP enzyme and its threonine mutants (T428A and T428K) to ascertain the significance of threonine in a PLP-independent catalytic mechanism and the ability of mutants to restore PLP cofactor binding. Also, the regulation of GP activity by several metabolic intermediates was assessed. MmGP exhibited a strong preference for long and branched oligosaccharides such as glycogen, with a K_M of 0.3 mM for phosphate and a half-saturation constant of 0.3 mg/ml for glycogen, values similar to those reported for bacteria and eukarya. Fluorescence spectroscopy demonstrated the absence of the PLP cofactor in the wild-type enzyme. Interestingly, the PLP binding capacity was partially recovered in the T428K mutant but not in the T428A mutant. Kinetically, the T428A mutant shows kinetic parameters similar to the wild-type enzyme, whereas the T428K mutant has no significant enzyme activity. Lastly, wild-type MmGP was inhibited by several metabolites, including NaPPi, fructose-6P, PEP, ADP, ADP-glucose, and UDP-glucose, and was activated by fructose-1,6-bisphosphate (FBP), showing a different regulation pattern from the one reported for other GP enzymes.
Acknowledgment: FONDECYT 1231263.

69. *In Vitro* Effects of Photodynamic Therapy and Epigallocatechin Gallate on the Nrf2 Pathway. Carmen Gloria Ili[1,2,3] (carmen.ili@ufrontera.cl), Daniela León[1,2,3,4], Claudio Tapia[1,2,5], Tamara Viscarra[1,2,6], Kurt Buchegger[2,3,4], Priscilla Brebi[1,2,3]. [1]Laboratory of Integrative Biology (LIBi), Centro de Excelencia en Medicina Traslacional (CEMT), Scientific and Technological Bioresource Nucleus (BIOREN), Universidad de La Frontera. Temuco, Chile. [2]Millennium Institute of Immunology and Immunotherapy. Santiago, Chile. [3]BMRC, Biomedical Reasearch Consortium-Chile. Santiago, Chile. [4]Departamento de Ciencias Básicas, Facultad de Medicina, Universidad de La Frontera. Temuco, Chile. [5]Carrera de Biotecnología, Facultad de Ciencias Agropecuarias y Medioambiente, Universidad de La Frontera. Temuco, Chile. [6]Biomedicine and Traslational Research Laboratory, Centro de Excelencia en Medicina Traslacional (CEMT),Universidad de La Frontera. Temuco, Chile.

Introduction: Photodynamic therapy (PDT) induces irreversible cytotoxicity by generating reactive oxygen species (ROS) through the interaction of an exogenous photosensitizer (PS), specific wavelength light, and intracellular oxygen (O_2). Resistant cells typically produce lower levels of PS and ROS, but this can be reverted by adding epigallocatechin gallate (EGCG), a green tea polyphenol, which enhances ROS production and cytotoxicity. The transcription factor NRF2 plays a pivotal role in orchestrating the cellular antioxidant defense by regulating the expression of genes involved in this response. Therefore, the objective of this study is to evaluate the cellular antioxidant response mediated by NRF2 in the context of PDT combined with EGCG.

Methodology: HaCaT (immortalized keratinocytes, sensitive to PDT) and A-431 (squamous cell carcinoma from skin, resistant to PDT) cell lines were used. Cells were exposed to PDT using 2 mM methyl aminolevulinate (MAL) as PS in combination with either 10 µM or 40 µM EGCG. A control group without EGCG was also included. After 4 h of incubation in darkness, the cells were irradiated with red light at a dose of 4 J/cm². Immediately afterward, the cells were collected for RNA extraction. Gene expression of the Nrf2 pathway was analyzed by RT-qPCR, using GAPDH and ACTB2 as housekeeping genes, determined by the $2^{-\Delta\Delta CT}$ method.
Results: In HaCaT cells subjected to PDT-EGCG treatment, a notable upregulation in the relative expression of genes associated with the Nrf2 pathway was detected, whereas Keap1 expression remained stable. In contrast, this upregulation was not observed in A-431 cells, despite the elevated expression of the gene encoding the glutathione reductase enzyme.
Discussion: This differential activation underscores the complexity of cellular antioxidant mechanisms and suggests that the Nrf2 pathway may play a critical role in mediating the sensitivity of cells to PDT-EGCG.
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70. Recombinant Production and Characterization of Diamine Oxidase (DAO) in *Escherichia coli* for Potential Histamine Intolerance Treatment. Rodrigo Iturra[1] (riturra2019@udec.cl), Amparo Uribe[2], José Martínez-Oyanedel[1], Maximiliano Figueroa[1]. [1]Laboratorio de Biofísica Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción. [2]Laboratorio de Enzimología, Facultad de Ciencias Biológicas, Universidad de Concepción.

Introduction: Histamine intolerance (HIT) is a condition where the body cannot adequately metabolize ingested histamine, leading to various symptoms such as migraines, stomach discomfort, and acne. This is primarily due to the reduced activity of the enzyme Diamine Oxidase (DAO), responsible for histamine metabolism. Recent studies suggest that oral supplementation with DAO derived from pig kidney extract can alleviate HIT symptoms. This study aims to produce the pig DAO enzyme recombinantly in *Escherichia coli* to develop a potential dietary supplement for HIT.
Materials and Methods: The gene encoding the DAO enzyme was designed and inserted into the pET22b expression vector to optimize expression in *E. coli* and to add a histidine tag for purification. *E. coli* BL21 cells were transformed, and multiple cultures were conducted to determine the optimal conditions for enzyme production. The enzyme was purified using chromatographic techniques, followed by an activity assay with histamine. Biophysical properties were characterized using circular dichroism and size-exclusion chromatography.

Results: The recombinant production of DAO in *E. coli* was successful, with positive results in enzyme activity assays and successful characterization of its biophysical properties. These results indicate that *E. coli* is a viable system for the recombinant production of DAO enzyme.
Discussion: The study demonstrates the feasibility of using *E. coli* for the recombinant production of the DAO enzyme, which could be further developed as a dietary supplement for managing histamine intolerance. The next steps will focus on optimizing production and evaluating the enzyme's effectiveness in clinical applications.
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71. Function of Ion Channels Glutamate Receptors (GLR) in Tomato Adaptation to Salt Stress. Andrea Jara[1] (constanzajaramanan@gmail.com), Oscar Arrey[1], Ricardo Cabeza[2], Erwan Michard[1]. [1]Laboratory of Plant Cell Sensing and Signalling (PCSS Lab), Instituto de Ciencias Biológicas, Universidad de Talca, Talca 3460000, Chile. [2]Plant Nutrition Laboratory, Department of Crop Sciences, Faculty of Agricultural Sciences, University of Talca, Chile.

Saline stress is a significant challenge for modern agriculture, especially in arid and semi-arid regions such as northern Chile. Soil salinity imposes osmotic and ionic stress on plants, which can lead to a substantial reduction in the yield of important crops like tomato (*Solanum lycopersicum* L.). Salt sensing, signaling and adaptation involve stress hormones such as ABA, JA, ethylene and cell second messengers, amongst which Ca^{2+} play major role. Originally identified in the nervous system of animals, where they are essential for synaptic transmission, GLRs are Ca^{2+}-permeable channels that are also found in plants where they have been involved in development, and responses to biotic and abiotic stress. Here, we challenge the hypothesis that GLRs play a specific role in salt stress adaptation. The main goal of the work was to investigate and quantify how the modulation of GLR activity affects the physiological response of tomato plants to saline stress. To address this question, experiments were conducted using tomato varieties *Solanum lycopersicum* cv. Money-Maker and microtom both grown in hydroponic conditions and *in vitro*. To challenge the role of GLRs in salt stress response, we treated plants subjected to NaCl with the GLR inhibitor AP-5. In hydroponic conditions, the following parameters were quantified: salt stress reporter gene expression, elemental composition, and plant growth. *In vitro* condition, we measured root growth, root length, number of lateral roots, and root hair. Our results clearly demonstrate a role of GLRs in tomato adaptation to saline stress. In conclusion, we discuss possible mechanisms involving GLRs in salt stress adaptation of tomato.
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72. Potential competing endogenous long non-coding RNAs in heart failure as epithelial-mesenchymal transition regulators of colon cancer genes. Jiménez-

Gallegos D.[1](danica.jimenez@ug.uchile.cl), Corrales WA.[1], Peñaloza-Otárola A.[1], Norambuena-Soto I.[1,3], Sigcho-Garrido F.[1], Torres VA.[1], Munita R.[1], Maracaja-Coutinho V.[1], Lavandero S.[1,2]. [1]Advanced Center of Chronic Diseases (ACCDiS), Facultad de Ciencias Químicas y Farmacéuticas & Facultad de Medicina, Universidad de Chile, Santiago, Chile & [2]Cardiology Division, UT Southwestern Medical Center, Dallas, Texas, USA. E-mail. [3]Department of Diabetes and Cancer Metabolism, Beckman Research Institute, City of Hope National Medical Center, Duarte, California 91010, USA.

Introduction: Cardio-oncology studies have revealed that chronic heart failure (CHF) affects cancer progression by a mechanism involving circulation factors. Newly systemic long non-coding RNAs (lncRNAs) have also been reported as CHF circulating biomarkers but have unknown pathophysiological relevance. LncRNAs may also act as competing endogenous RNAs (ceRNAs) and are known to be involved in the regulation of epithelial-mesenchymal transition (EMT) genes in colon cancer.
Aim: To identify potential HF derived ceRNAs as cancer progression regulators in epithelial-mesenchymal transition.
Methodologies: Differential expression (DE) analysis of public RNA-seq datasets of left ventricle and blood HF individuals**,** and colon cancer (CC) tumor samples were assessed to obtain HF associated lncRNAs (Log2FC > 0,5, p-value adj < 0,05) and cancer target mRNAs (Log2FC > 1,5, p-value adj < 0,05). ceRNA networks were identified by cross-referencing lncRNA candidates with an in-house developed human interaction network built based on experimentally validated lncRNAs-miRNAs and miRNAs-lncRNAs interactions. Co-expression of lncRNAs and EMT mRNA targets was evaluated by Spearman correlation analysis. Finally, the ceRNA *SILC1* 1790 kb sequence was cloned and overexpressed by lipofectamine 2000 transfection in colon cancer cells DLD-1 for 48h. qRT-PCR and Western blot assessed EMT biomarker expression levels.
Results: We identified 16 DE lncRNAs in common between HF left ventricle and blood. Among them, 4 ceRNAs were predicted to target miRNAs, which regulate mRNAs related to genes associated with epithelial-mesenchymal transition and mitotic cell cycle processes. *CiclinD1*, *CDH1*, and *VIM* were identified as downregulated by *SILC1* 1 ug overexpression.
Conclusion: HF patients' blood ceRNAs could regulate colon cancer progression coding genes, but *SILC1* complete transcript overexpression regulates epithelial as well as mesenchymal biomarkers.
Acknowledgments: This work was supported by Agencia Nacional de Investigación y Desarrollo (ANID) through the FONDECYT 1211731 (VM), FONDAPs 15120011, 1523A0008 (SL, VM, RM, IN), FONDECYT Iniciación 11230662 (RM), FONCECYT Postdoctoral 3210496 (IN) and PhD fellowship 21210478 (DJ).

73. Cloning of the polymorphic Cytotoxin Associated Gene A (*CagA*) gene of *Helicobacter pylori* for *in vitro* studies. Samuel Jorquera (samuel.jorquera@ing.uchile.cl), Roxana González-Stegmaier, Constanza Cárcamo,

Joaquín Reyes, Franz Villarroel-Espindola. Translational Medicine Unit. Fundación Arturo López Pérez. Santiago, Chile.

Introduction: *Helicobacter pylori* (*HP*) is strongly linked to gastric cancer. *HP* has different virulence genes, including *CagA,* which is polymorphic and encodes a protein responsible for the pathological changes of the gastric epithelium. CagA protein has a phosphorylation motif called EPIYA (Glu-Pro-Ile-Tyr-Ala) which has evolved based on the number of repeated domains and by its surrounding peptides. This work aimed the construction of recombinant constructs for *in vitro* studies of *HP*-induced damage based on the isolation of *CagA* gene from well-known polymorphic *HP* strains.
Materials and Methods: *HP* strains were obtained from the ATCC (J99, 26695 and NCTC11637). The whole *CagA*-codifying gene (~3.5 Kbp) was cloned into the expression system "pF4 CMV Flexi vector". Additionally, ΔEPIYA-variants and isomorphic variants were generated by site-directed mutagenesis. AGS, Kato-III and NCI-N87 cell lines were transfected to evaluate CagA expression.
Results: For each strain, 2 to 3 recombinant plasmids were generated and characterized by PCR and restriction assay. As expected, all clones had a recombinant plasmid of 7.3 – 7.5 Kbp and the restriction sites SwaI and AvrII, which are specific for the inserted gene. Plasmid containing ΔEPIYA-variants were characterized by PCR and it showed 1Δ, 2Δ and 3Δ of EPIYA-C domain, lacking 318, 387 and 578 bp compared to the parental gene, respectively. All clones were sequenced and confirmed by *in silico* analysis. Immunoblot assays demonstrated the expression of each recombinant construct.
Discussion: We cloned several polymorphic variants of CagA from *HP,* and they can be used in the study of gastric carcinogenesis mediated by EPIYA polymorphisms.
Acknowledgment: ANID-FONDECYT 1221415, FALP-LMT-2024.

74. New Insights into the Mechano-Structural Properties of the Adhesion System of the Oral Pathogen Porphyromonas gingivalis. Josefa Nuñez-Belmar[1,2](josefa.nunezb@mayor.cl), Luis Alberto Valverde Fernández[4], Juan P. Cárdenas [3,5], Richard Charles Garratt[4], Jaime Andrés Rivas Pardo[3,5]. [1]Doctorado en Genómica Integrativa, Universidad Mayor, Santiago, Chile. [2]Escuela de Odontología, Facultad de Medicina y Ciencias de la Salud, Universidad Mayor, Santiago, Chile. [3]Centro de Genómica y Bioinformática, Universidad Mayor, Santiago, Chile. [4]São Carlos Institute of Physics, USP, São Carlos, SP, Brazil, [5]Escuela de Biotecnología, Facultad de Ciencias, Tecnología e Ingenieria. Universidad Mayor, Santiago, Chile.

Introduction: Periodontitis is a chronic, immuno-inflammatory disease characterized by the loss of tooth-supporting tissues, ultimately leading to tooth loss. *Porphyromonas gingivalis* plays a crucial role in causing dysbiosis, disrupting host immunity, and perpetuating inflammation in periodontitis. The first step of host infection is the initial adhesion of fimbriae (FimA) to gingival epithelial cells. FimA are filamentous structures anchored to the external

bacterial membrane. Previous studies have shown a correlation between the phenotypes of FimA and their adhesion capacity to the host epithelium. Currently, there are few structural studies of FimA genotypes. This study aims to describe the structural differences between FimA genotype I (from an avirulent strain) and FimA genotype IV (from a virulent strain).
Materials and Methods: FimA genotype I and IV were isolated and purified after being expressed in *E. coli* Rosetta (DE3). The bands of each protein were confirmed by mass spectrometry. Circular dichroism (CD) and Size-Exclusion Chromatography coupled with Multi-Angle Light Scattering (SEC-MALS) experiments were conducted.
Results: The CD spectra analysis revealed significant structural differences in the secondary structure between FimA genotype I and genotype IV (p-value 7.49×10^{-7}). The SEC-MALS analysis indicated that both are monomeric proteins, with molecular weights of 38.32 kDa for type I and 37.97 kDa for type IV.
Discussion: The differences suggest that FimA type I is likely to have a higher proportion of alpha-helices and potentially greater stability compared to type IV. Our data correlate and enrich the structural information of both proteins. This contributes to our understanding of the mechanical behavior of FimA regarding adhesion to the host and establishes a foundation for further characterizations that are ongoing, including protein-ligand interaction studies and atomic force microscopy.
Acknowledgment: This work was supported financially by ANID through FONDECYT project 1221064 (JARP). The Genomics Integrative PhD Program at Universidad Mayor and the UNU-BIOLAC fellowships support JNB.

75. Meta-analysis of the drought and Nitrogen responses in *Solanum lycopersicum* reveals an important molecular crosstalk and putative transcription factors integrating these responses. Diego Landaeta-Sepúlveda [1,2,3] (diego.landaeta@mayor.cl), Nathan R. Johnson[1,2], José M. Álvarez[2,4], Elena A. Vidal[1,2]. [1]Centro de Genómica y Bioinformática, Universidad Mayor, Chile. [2]ANID-Millennium Science Initiative Program-Millennium Institute for Integrative Biology (iBio). [3]Doctorado en Genómica Integrativa, Universidad Mayor. [4]Centro de Biotecnología Vegetal, Universidad Andrés Bello.

Introduction: Water and nitrogen (N) availability are among the main factors determining plant growth and productivity. Thus, it is of paramount importance to understand how plants sense and respond to these signals, in a scenario of increasing drought periods and massive use of N-based fertilizers. Individual molecular mechanisms of drought- or N-responses are well-studied in Arabidopsis and relevant crops, however the response to their combined effect is less understood. Notably, an overlap between Water- and N-responsive genes has been reported in *Arabidopsis* and rice, suggesting common regulatory factors and possibly involving a crosstalk mediated by the abscisic acid signaling pathway.
Materials and Methods: To identify common-genes and transcription factors (TFs) involved in drought- and N-responses in tomato, we performed a meta-analysis of various transcriptomic studies, covering different tissues, cultivars, and developmental stages of

tomato under drought or under varying N availability conditions. We identified differentially expressed genes (DEGs) with consistent responses across conditions for each treatment and used the GENIE3 algorithm for gene regulatory network (GRN) inference to find potential TFs integrating both responses.
Results: We found a significant overlap between drought and N-responsive genes in tomato, with many common genes showing inverse responses (induced by drought and repressed by N, or vice versa). The GRN models identified several putative integrator TFs, including AREB1, which is associated with the ABA response.
Discussion: Our study reveals substantial crosstalk between drought- and N-responses in tomato. Analysis of the common DEGs suggests a trade-off between stress responses and growth, potentially mediated by an ABA pathway controlled by AREB1.
Acknowledgments: ANID-FONDECYT1211130/1210389, ANID-iBio-ICN17_022, FOVI230159, and ANID-Beca de doctorado nacional 21230939.

76. Functional investigation of histones H3 and H4 in mitochondria. Scarleth Larraín Goicovich (scarlethlarrain@gmail.com), Alejandra Catenaccio, Cheril Tapia, Alejandra Loyola. Department of Basic Sciences, Faculty of Medicine and Sciences, Universidad San Sebastián, Santiago de Chile, Laboratory of Epigenetics and Chromatin, Fundación Ciencia & Vida.

Introduction: Histones are small, highly conserved basic proteins in eukaryotic cells, consisting of globular domains and N-terminal tails. These proteins undergo various post-translational modifications (PTMs), which play crucial roles in enabling histones to perform diverse functions, particularly in chromatin structure by packaging and organizing DNA. Besides their well-known function in chromatin, recent research has uncovered histones in an unexpected cellular location: mitochondria. Building upon this discovery, this study investigated the presence and potential function of histone H3 in mitochondria.
Materials and Methods: This investigation utilized HeLa cells and mESC (mouse embryonic stem cells) cells for subcellular fractionation, microscopy, cell digestion, mass spectrometry, and cellular stress assays.
Results: The presence of histones H3 and H4 was detected in mitochondrial fractions from various cell lines, with a potential interaction at the outer mitochondrial membrane zone, and their post-translational modifications were determined.
Discussion: These findings confirm the presence of histones H3 and H4 in mitochondria and suggest a possible non-canonical role linked to mitochondria. This brings us closer to understanding the diverse functions that histones may have within mitochondria.
Funding: Fondecyt Regular 1240409, Centro Ciencia & Vida FB210008, Financiamiento Basal para Centros Científicos y Tecnológicos de Excelencia.

77. Methylome transcriptional regulatory network activated by copper in *Enterococcus faecalis*. Gabriel Gálvez[1,2], Víctor Aliaga-Tobar[1,2], Mauricio Latorre[1,2,3]

(mauricio.latorre@uoh.cl).[1]Laboratorio de Bioingeniería; Instituto de ciencias de la ingeniería; Universidad de O'Higgins, Rancagua, Chile. [2]Centro de biología de sistemas para el estudio de comunidades extremófilas de relaves mineros (SYSTEMIX), Universidad de O'Higgins, Rancagua, Chile. [3]Laboratorio de bioinformática y expresión génica, INTA, Universidad de Chile, Santiago, Chile.

Introduction: Transcriptional regulation is commonly associated with transcription factors, but another important layer is methylation, where DNA bases are chemically modified to modulate the affinity of the transcriptional machinery. In this study, using the bacterium Enterococcus faecalis as a model, information from the copper response methylome was integrated into a transcriptional factor regulatory network (TRN) for the first time, with the aim of understanding the combined configuration of both gene regulation mechanisms in response to the metal.
Materials and Methods: *Enterococcus faecalis* OG1RF strain (Id 474186). Copper treatment: 0.5 mM $CuSO_4$, 3 hours). Sequencing: Methylome (SMRT and bisulfite), Transcriptome (RNAseq). Bioinformatics: Bowtie2 mapper, SMRT Analysis tool, intersectBed tool, MEME tool, BEDTools, Cytoscape, TRNT from Latore et al. 2024 (PMID: 24382465).
Results: We identified a total of 918 methylation patterns across the promoters of *E. faecalis* (55% of the total genome). Interestingly, 20% of these methylation patterns changed in response to copper exposure, indicating a significant epigenetic response induced by the metal. Integrating this information into a transcriptional regulatory network of E. faecalis allowed us to generate an integrated model of regulation between transcription factors and promoter methylation. The integrated network comprises a total of 59 genes, of which 16 increased and 12 decreased their mRNA levels in response. We identified two types of regulatory modules: one highly connected module covering more than 70% of the network components, and four isolated independent modules, within which the copper homeostasis mechanisms (*cop* operon) were identified.
Discussion: The integration of different transcriptional regulatory mechanisms into an integrated model opens a new perspective on global gene regulation induced by metals in bacterial species.
Acknowledgment: CMM ACE210010; FB210005; ANID Millennium CRG ICN2021_044; ANILLO ANID ACT210004; BioSAV UOH; FONDECYT 1230194.

78. The effect of the chaperone Trigger Factor on the dimerization and structural dynamics of the DNA-binding domain of the human transcription factor FoxP1. Jesús Lira Gerardo (jesus.lira@ug.uchile.cl), Rodrigo Rivera, 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), characterized by dimerization via segment exchange. Several studies have shown that the presence of disordered regions affect the FKH's dimerizarion 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 protein exchange processes.
Materials and Methods: The wild-type monomer FKH of FoxP1 was subjected to analysis using size exclusion chromatography to determine the effect of TF on FKH's dimerization properties. In addition, single-cysteine mutants of FKH of FoxP1 were generated and purified, labeled with Bodipy as probe, and the structural flexibility was investigated using fluorescence anisotropy at single-molecule level.
Results: The presence of the chaperone TF decreases the dimer population in equilibrium conditions. Additionally, while the flexibility of FoxP1 is heterogeneous, exhibiting differences at the local level when comparing helix H2 with helix H5, the presence of TF stabilizes a disordered-like monomer in a cooperative manner.
Discussion: These findings suggest that a chaperone such as TF may contribute to the stabilization of the monomeric state of FoxP1 under cellular conditions, regulating their gene-expression activity.
Acknowledgment: Fondecyt 11200729 / Fondecyt 1231276 / Fondequip EQM200202.

79. Metaphors, a case study in a biochemistry and biophysics laboratory, Universidad de Chile. Flavia I. Livacic-Rojas (flavialivacicr@gmail.com). Biochemistry Laboratory. Biochemistry and Molecular Biology Department. Faculty of Chemistry and Pharmaceutical Sciences, Universidad de Chile.

A metaphor is a figure of speech that describes an object or action in a way that is not literally true but helps to explain an idea or make a comparison. In science, metaphors can help to illustrate abstract concepts by drawing parallels to tangible objects or processes that people are related to.
This study is a mixed (qualitative and quantitative) research of how metaphors are used in biochemistry and biophysics. It started as an ethnographic study as participant-observer of metaphors utilized in the Biochemistry Laboratory, Faculty of Chemistry and Pharmaceutical Sciences, Universidad de Chile. It was followed by a classification and characterization work, supported by literature review on the historical use and journey of certain metaphors in biochemistry and biophysics.
The forty-seven (47) words or expressions captured throughout this participant observation were listed and tagged as "corpus", which is constituted of five variables: as a role, devices and tools, cuisine, personification, and miscellaneous. Two metaphors were selected to be reviewed in detail, based on their relevance and extensive use in this specific biochemistry and biophysics laboratory. The selected terms are "chaperone" to refer to an accompanying protein, and "chameleonic" which is utilized for a protein that changes its conformation and is also referred to as "metamorphic".

This initial study compiled several metaphors and revised the two most frequent ones. The resulting corpus could be a starting point for the development of a compendium of metaphors for the biochemistry and biophysics areas. Further studies such as large surveys and additional literature reviews would be required as a continuation of this endeavor.

Sponsored by: Dr. Christian A.M. Wilson.

80. Analysis of the antiviral activity of NS1/Rep-derived endogenous parvoviral elements. Pablo Lobos-Ávila (p.lobosvila@uandresbello.edu), Pablo Abufom and Gloria Arriagada. Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad Andrés Bello, Santiago, Chile.
Introduction: Endogenous parvoviral elements (EPV) represent ancestral parvoviral infections in a host germ line present in extant species. In *Octodon degu* (degu), the *EPV-Dependo.43ODegus* locus encodes an NS1/Rep-derived protein named DeRep. *In vitro* DeRep has antiviral activity against the minute virus of mice (MVM). DeRep localizes in the cell nucleus and directly binding MVM DNA. In degu, we also found a smaller protein (DeRepS), corresponding to the C-terminal portion of DeRep. In *Chinchilla lanigera* (chinchilla), we identified other NS1/Rep-derived EPVs with intact open reading frames called ClanRep. Here, we evaluate the localization and antiviral activity of these NS1/Rep EPV-derived proteins.
Materials and Methods: The coding sequences for DeRep, DeRep mutated into its nuclear localization signal (DeRepΔNLS), DeRepS, and ClanRep were cloned with a FLAG epitope into viral vectors. Stable NIH3T3 cell lines expressing each of these proteins were generated using viral vectors. The localization of each protein was analyzed by immunofluorescence. All stable cells were challenged with serial dilutions of MVM to assay antiviral activity.
Results: In non-infected cells FLAG-DeRep, FLAG-DeRepS and FLAG-ClanRep localize to the cell nucleus, while FLAG-DeRepΔNLS, localized to the cytoplasm. Like DeRep, DeReps and ClanRep can protect cells against MVM infection. Surprisingly, DeRepΔNLS also protects cells of MVM infection.
Discussion: Our data suggest a conserved function among EPVs derived of NS1/Rep that have open reading frames in the antiviral activity against an exogenous protoparvovirus. We still need to test if all these EPV bind viral DNA as DeRep does. Since FLAG-DeRepΔNLS is cytosolic and also protect against MVM, we are currently testing if its localization changes upon infection.
Acknowledgment: FONDECYT 1220480, Anillo ATE 220007.

81. Insulin regulates MUL1 expression in cultured skeletal muscle cells. Erik Lopez-Gallardo[1], Ignacio Norambuena-Soto[1], Marioly Müller-Sobarzo[1,2], Valentina Parra[1], Sergio Lavandero[1,2,3]. [1]Advanced Center for Chronic Diseases (ACCDiS), University of Chile, Santiago, Chile. [2]Faculty of Medicine, University of Chile, Santiago, Chile. [3]Cardiology Division, University of Texas Southwestern Medical Center, Dallas, Texas, USA.

Introduction. Insulin regulates mitochondrial dynamics and function by the protein kinase Akt, generating an elongated mitochondrial phenotype and stimulating mitochondrial oxidative metabolism. **The** mitochondrial E3 ligase MUL1 ubiquitinates Akt and the mitochondrial fusion protein Mfn2, targeting for its proteasomal degradation. MUL1 also increases Drp1-induced mitochondrial fragmentation and decreases insulin-induced mitochondrial metabolism. Furthermore, MUL1 expression significantly increases under insulin resistance condition. Thus, MUL1 can potentially regulate insulin sensitivity. However, the role of MUL1 on the insulin signaling pathway is poorly explored. This work was aimed to evaluate the effect of insulin on MUL1 expression in cultured skeletal muscle cells and cardiomyocytes at different times.
Results. The results showed that the Drp1 and Mfn2 expression level increased in L6 myoblasts under insulin stimulation until 4 h post-stimulation. However, we observed that insulin did not change MUL1 protein expression in cultured skeletal muscle cells.
Conclusion. Our findings suggest that insulin could regulate the MUL1 activity, but not the expression, in cultured skeletal muscle cells. Thus, MUL1 could be a novel component of the insulin signaling pathway in skeletal and cardiac muscle, but further studies are required to test this hypothesis.
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82. HuR and hnRNPK are IRES-transacting factors for the MMTV IRES. Dafne Mendonça[1], Marcelo López-Lastra[1]. [1]Laboratorio de Virología Molecular, Instituto Milenio de Inmunología e Inmunoterapia, Departamento de Enfermedades Infecciosas e Inmunología Pediátrica, Escuela de Medicina, Pontificia Universidad Católica de Chile, Marcoleta 391, Santiago, Chile.

Introduction: The Mouse Mammary Tumor Virus, a beta-retrovirus that exists in both exogenous (MMTV) and endogenous (*Mtv*) forms, induces mammary carcinoma in mice and has been associated with breast cancers in humans. The MMTV full-length mRNA harbors an IRES within its 5'UTR that is functional in rabbit reticulocyte lysates, *Xenopus laevis* oocytes, and several cell types, including HEK29T cells. The activity of the MMTV-IRES differs in different cellular backgrounds, suggesting that optimal translational activity of the MMTV IRES requires IRES-transacting factors (ITAFs). In agreement with this possibility, hnRNPA1 has been reported to modulate MMTV IRES activity in HEK293T cells. This work aimed to determine if hnRNPK and HuR, described ITAFs for other retroviral IRESs, affect the MMTV IRES activity.
Material and Methods: A dual luciferase bicistronic vector harboring the MMTV mRNA 5'leader, RNA region which harbors MMTV IRES activity in its intercistronic space, was used to evaluate the role of hnRNPK and HuR as regulators of the MMTV IRES.

Results: We provide evidence that, in HEK293T cells, hnRNPK and HuR stimulate the activity of the MMTV IRES. In addition, results suggest that asymmetrical dimethylations of arginine residues (ADMAs) impact the effect of hnRNPK over the MMTV IRES.
Discussion: HnRNPK and HuR, proteins associated with human breast cancer, are ITAFs for the MMTV IRES. Moreover, the protein arginine methyltransferase 1 (PRMT-1) induced asymmetrically dimethylation (ADMA) of hnRNPK impacts the protein's ability to stimulate MMTV-IRES.
Acknowledgment: Beca Doctorado ANID 21200983, FONDECYT 1210736, and the Iniciativa Cientifica Milenio (ICN09_016/ICN 2021_045).

83. 17β-Estradiol induces a senescent phenotype in human pulmonary artery smooth muscle cells. Camila López-Torres[1,2,3](camila.lopez.t@ug.uchile.cl), Francisco Sigcho[2,3], Jaime Riquelme[1,3], Valentina Parra[2,3,4]. [1]Laboratorio de Farmacoterapia Cardiovascular, Departamento de Química Farmacológica y Toxicológica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile. [2]Laboratorio de Diferenciación Celular y Metabolismo, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile. [3]Advanced Center of Chronic Diseases (ACCDiS), Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile. 4Systems Biology Center for the Study of Extremophile Communities from Mining Tailings (SYSTEMIX), O'Higgins University, Rancagua, Chile.

Introduction: Pulmonary arterial hypertension (PAH) is an arteriopathy that exhibits remodeling in pulmonary arteries and arterioles. In particular, pulmonary artery smooth muscle cells (PASMC) undergo a phenotype change associated with metabolic reprogramming, which ultimately leads to arterial remodeling. One of the pathogenic mechanisms involved is the emergence of a subpopulation of senescent cells that are responsible for contributing to remodeling through the secretion of different molecules. Elevated levels of 17β-estradiol in patients with idiopathic PAH have been associated with increased risk and severity of this disease. 17β-estradiol promotes PASMC proliferation; however, it is unknown how 17β-estradiol mediates its effects, with one possible mechanism being cellular senescence. To date, 17β-estradiol has been described to be able to induce senescence in other cell types, but whether it does so in PASMCs is unknown. Therefore, we sought to determine whether estradiol can induce cellular senescence in hPASMCs.
Methodology: Human PASMC (hPASMC) were treated with 100 nM of 17β-estradiol for 72 h and different markers of cellular senescence were evaluated. β-galactosidase activity was determined using a β-galactosidase staining kit; protein content of p16, p21 and phosphorylated retinoblastoma (pRb) was assessed by Western blot, whereas mRNA levels of p16, p21 and p53 were measured by qRT-PCR. DNA damage (γH2AX) and nuclear morphology was determined by immunofluorescence.

Results: 17β-estradiol increased β-galactosidase staining but did not increase the levels of p16, p21, p53, or pRb by either Western blot or qRT-PCR. However, immunofluorescence of γH2AX showed increased foci in the nucleus and an increase in the nuclear perimeter.
Discussion: These results suggest that 17β-estradiol induces cellular senescence in a subpopulation of hPASMC, which could contribute to PAH pathogenesis. Future studies should explore targeted therapies to mitigate this effect.
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84. The Role of ADAR1 in the Regulation of DNA Damage Response in Breast Cancer Cells. Lorena Maldonado (lm_adar_lgfc@udd.cl) [1,2], Isidora Solar[2], Lorena Abarzúa-Catalan[2], Eduardo Peña[3], Ricardo Armisén[2]. [1]Programa de Magíster en Ciencias Químicas y Farmacéuticas, Facultad de Ciencias de la Salud, Universidad Arturo Prat. [2]Laboratorio de Genómica Funcional del Cáncer, Facultad de Medicina Clínica Alemana, Universidad del Desarrollo. [3]Centro de investigación en Medicina de Altura (CEIMA), Universidad Arturo Prat.

Introduction: Breast cancer (BC) is the most prevalent cancer in women, with a substantial socioeconomic impact. ADAR1 (adenosine deaminase acting on RNA 1) is overexpressed in various cancers, including BC. Several studies suggest ADAR1's involvement in regulating key proteins in the DNA damage response, raising the possibility of ADAR1's role in modulating the efficacy of PARP inhibitors. This study aimed to determine the protein expression of BRCA1, MRE11, and phospho-γ-H2AX in the MDA-MB-231 breast cancer cell line with modified ADAR1 expression.
Materials and Methods: MDA-MB-231 breast cancer cells with loss-of-function (KD) and gain-of-function (OE) modifications of ADAR1 were utilized. Protein levels of MRE11 and BRCA1 (involved in homologous recombination DNA repair) and ADAR1 were measured by western blot (WB). Additionally, the phosphorylation of y-H2AX as DNA damage marker were measured by WB too. Cell viability was measure with MTS.
Results: A significant increase in BRCA1 and MRE11 protein expression was observed in MDA-MB-231 cells with ADAR1 KD, while the opposite effect was seen with ADAR1 OE. Additionally, γ-H2AX phosphorylation decreased in the ADAR KD condition. Preliminary experiments indicate that ADAR1 KD cells are more sensitive to a PARP inhibitor.
Conclusion: These results demonstrate that ADAR1 overexpression can regulate the expression of BRCA1 and MRE11, proteins involved in DNA repair mechanisms. Consequently, a decrease in the DNA damage marker phospho-γ-H2AX was observed in the KD condition.
Acknowledgment: Fondecyt 1221436.

85. miR-15b Modulation of p53/p21cip and p16ink4A Pathways in Cardiomyocyte. Mancilla Georthan[1,2,3,4] (endlos@ug.uchile.cl), Oyarzun Ingrid[3,4], Castro Pablo[3,4], Quiroga Clara[3,4], Pedrozo Zully[1,2,4], Verdejo Hugo[3,4]. [1]Laboratorio de Mecanotransducción de señales cardiovasculares. Departamento de Fisiología, Facultad de Medicina, Universidad de Chile. [2]Instituto de Ciencias Biomédicas (ICBM), Facultad de Medicina, Universidad de Chile. [3]Laboratorio de Señalización Cardiovascular, División de Enfermedades Cardiovasculares, Facultad de Medicina, Pontificia Universidad Católica de Chile. [4]Advanced Center for Chronic Diseases (ACCDIS), Santiago, Chile.

Introduction: miR-15b, through its interaction with the RNA-induced silencing complex (RISC), is known to negatively regulate multiple molecular targets associated with the cell cycle, such as cyclin D and cyclin E, leading to cell cycle arrest and induction of apoptosis. However, its role in post-mitotic cells like cardiomyocytes remains unclear. While elevated levels of miR-15b have been associated with detrimental effects in models of acute cellular injury, their impact in chronic conditions is poorly understood. In this study, we aim to investigate the potential role of miR-15b in the regulation of senescence.
Materials and Methods: We evaluated classical markers of senescence, including the activation of the p53/p21cip and p16Ink4a pathways and the phosphorylation of the γH2A.X histone in neonatal rat cardiomyocytes transfected with miR-15b mimic and antagomiR-15b.
Results: Transfection with antagomiR-15b significantly decreased the expression of both p53/p21cip and p16Ink4a, while transfection with a miR-15b mimic significantly upregulated the expression of these pathways.
Discussion: miR-15b appears to regulate two independent pathways involved in cell cycle control and senescence, consistent with its reported role in various cancers. While it is hypothesized that miR-15b may influence the p53/p21cip pathway through interactions with lncRNA Hotair and Malt1, the mechanism by which it regulates p16Ink4a remains unknown. These findings suggest that antagomiR-15b could play a protective role by counteracting the induction of senescence.
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86. Palmitate and L-NAME induce a senescent phenotype in A7r5 cells at a protein and morphologic level. Javiera Martínez Bilbao[1,2](ivanea.martinez@gmail.com), Fernanda Sanhueza Olivares[1], Andrea Mella Torres[1], Francisca Valenzuela Arce[1], Mario Chiong Lay[1]. [1]Advanced Center for Chronic Diseases, Faculty of Chemical and Pharmaceutical Sciences, University of Chile. [2] PhD in Biochemistry Program, Faculty of Chemical and Pharmaceutical Sciences, University of Chile.

Introduction: Heart failure with preserved ejection fraction (HFpEF) is an age-related disease. It has been proposed that one of the main characteristics of HFpEF is vascular dysfunction. We propose that vascular smooth muscle cells (VSMCs) senescence plays a

significant role in HFpEF-associated vascular dysfunction. HFpEF mouse model involves inducing nitrosative stress in the heart through a combination of a high fat diet and L-NAME. Therefore, we aimed to evaluate whether the treatment of A7r5 VSMC cultures with palmitate and L-NAME, stimuli that induce HFpEF *in vivo*, promotes cell senescence.
Materials and methods: Toxicity levels of L-NAME and palmitate were determined using dose-response assays, assessing cell viability through cell counting, MTT assays, and PI permeability via flow cytometry. Senescence was evaluated by assessing senescence-associated β-galactosidase activity, IL-6 release into the cell culture media by ELISA, and p53 expression through immunofluorescence. Cell size was determined in microscopy images by free-hand drawing of the cell limits, measuring perimeter and area in ImageJ.
Results: Non-toxic concentrations of L-NAME and palmitate, following 72 hours of stimulation, were 5 mM and 1 nM, respectively. These treatments increased A7r5 VSMC cell size and area, increased senescence-associated β-gal activity, IL-6 release and p53 levels.
Discussion: The model generated by the treatment of A7r5 VSMC with 1 nM palmitate and 5 mM L-NAME for 72 hours triggers a senescent-like phenotype. This suggests a role for senescence in vascular dysfunction associated with HFpEF, proposing new pharmacological targets for treatment of this disease.
Acknowledgments: This work was supported by the Agencia Nacional de Investigación y Desarrollo (ANID) Chile (FONDECYT 1220392 and FONDAP 1523A0008) and ANID-Subdirección de Capital Humano/Doctorado Nacional/2023/Folio 21232137.

87. Effect of water deficit on the expression of quercetin biosynthesis related genes in *Eucalyptus spp.* José N. Medina[1,2] (josenmedinam@gmail.com), Raúl Herrera[3], David Ramírez[2], Marta Fernández[1]. [1]Laboratorio Genómica Forestal, Centro de Biotecnología, Universidad de Concepción, Concepción, Chile. [2]Departamento de Farmacología, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile. [3]Laboratorio de Fisiología Vegetal y Genética Molecular, Instituto de Ciencias Biológicas, Universidad de Talca, Chile.

Introduction: *Eucalyptus spp*. is one of the most important species in the forestry industry both nationally and internationally, due to its wood quality and rapid growth. However, climate change has affected its productivity and survival capacity due to biotic and abiotic stress environments, such as drought. Several studies have shown that the flavonoid biosynthetic pathway plays a fundamental role in plant tolerance to different types of stresses. A growth chamber experiment was designed to assess the effect of water deficit on 5 different genotypes of *Eucalyptus spp.* and evaluate the relationship between water stress response, transcript accumulation of quercetin biosynthesis related genes and the quercetin accumulation in leaves of those genotypes.
Materials and Methods: Five *Eucalyptus spp*. genotypes of juvenile plants were maintained for 11 days under no irrigation. A group of three plants per genotype was dairy irrigated as a

control group. Xylematic potential and leaf water content was assessed to determine water deficit response. To detect and quantify quercetin, the UHPLC-QTOF-MS technique was used. Transcript accumulation of the quercetin molecular pathway related genes was quantified by real-time qRT-PCR.
Results: There were significant differences among genotypes in response to water deficit treatment. Quercetin metabolites and gene expression were significantly regulated under water deficit treatments.
Discussion: These results suggest that the quercetin pathway may play an important role in water deficit stress response in *Eucalyptus spp*.
Acknowledgments: Proyecto Anillo ATE220043.

88. Senescence in rat aorta vascular smooth muscle cells induced by iNOS. Andrea Mella Torres (andrea.mella@ug.uchile.cl), David Silva, Erik López, Liliana Espindola, Francisca Valenzuela Arce, Javiera Ivanea Martinez, Sergio Lavandero, Mario Chiong. 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, damaged molecules accumulate that cause oxidative stress, 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 no evidence linking iNOS directly to the generation of senescence in vascular smooth muscle cells (VSMCs).
Objective: 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 adenoLac Z or adenoGFP, and adenoiNOS, and they were purified by a discontinuous CsCl gradient. A7r5 cells, passages 5-12, were transduced or not with adenoLac Z or adenoGFP (control) or adenoiNOS, using different multiplicities of infection (MOI) for 72 hours. They were then evaluated by western blotting and immunofluorescence. The cytotoxicity of overexpression was evaluated by measuring cleaved Caspase-3. Senescence was assessed by p16, p21, and phospho-Retinoblastoma (pRb) by western blot, pRb and γ-H2A.X by immunofluorescence, and β-galactosidase assay at 0, 3, 5 and 7 days post-transduction.
Results: Overexpression of iNOS did not induce apoptosis but increased p16, p21, and γH2A.X levels, while pRb levels decreased in VSMCs at 3 days post-transduction. Moreover, beta-gal activity increased in VSMCs at 5 days post-transduction.
Discussion: Our results suggest that iNOS induces senescence in VSMCs *in vitro*. However, further work is required to confirm these preliminary results.
Acknowledgment: This work was supported by Fondecyt 1220392, FONDAP 1523A0008 and Beca de Doctorado Nacional ANID Chile 21202230.

89. Optogenetic Control of Piezo1 Channels with Photoisomerizable Amino Acids. Hans Menares, Reyes Rachel and Ignacio Díaz-Franulic. Laboratorio de Biofísica, Centro de Bioinformática y Biología Integrativa CBIB, Universidad Andres Bello.

Introduction: Mechanosensitive ion channel Piezo1 is fundamental in the perception of mechanical stimuli and their conversion into electrochemical signals in cells. A detailed understanding of activation mechanisms and conformational changes of these channels are crucial for understanding channel function in health and disease. Here we unveil gating hotspots capable of absorbing mechanical energy and translate it into channel opening by using a photoisomerizable non-canonical amino acid that allows light-dependent activation of Piezo1.
Materials and Methods: We replaced individual codons in the mouse PIEZO1 gene with an AMBER stop codon (TAG), one by one, and used an orthogonal tRNA/tRNA synthetase system from Methanosarcina mazei to incorporate the non-canonical photoisomerizable amino acid Phenylalanine-4-Azobenzene (Phe 4-Azo) into the nascent polypeptide chain of Piezo1 expressed in HEK Piezo1 -/- cells. Ca2+ imaging assays were performed using Fluo-4 and 360-485nm LED as light source.
Results: The increase in Fluo-4 fluorescence signal after UV or blue light stimulation in cells transfected with AMBER Piezo variants incubated with Phe 4-Azo, but not in non-transfected or non-UAA supplemented cultures, suggests that several residues in the pore and blade domains are critical components of the channel's tension-sensing pathway, effectively rendering Piezo1 channels light-gated.
Discussion: The 9-angstrom change in the azobenzene sidechain length after photoisomerization is sufficient to activate the channel, suggesting that the mechanical stimulus is transmitted through nanoconfined domains where small movements are efficiently conveyed from the channel periphery to the pore domain. Extending our analysis to more distant channel domains would allow us to develop a more detailed understanding of membrane tension sensing and transmission in the Piezo1 channel.

90. Peptide Development for the blocking the Isopeptide bonds on Spy0128 protein from *Streptococcus pyogenes*. Michelle Mendoza Becerra (michelle.mendoza@mayor.cl), Tomás Hermosilla Peña, J. Andrés Rivas Pardo. Mechano Biology Group, Microbe Genomics lab, Center for Genomics and Bioinformatics, Universidad Mayor, Santiago, Chile.

Introduction: *Streptococcus pyogenes* is a bacterium that causes many diseases, such as tonsillitis, pharyngitis, impetigo, and necrotizing fasciitis. These bacteria adhere to host cells through their pili, filamentous structures formed by hundreds of pilin protein repeats. These proteins must withstand major mechanical challenges without unfolding and remain anchored to the host. The key structural component that confers mechanical strength to pilins is internal isopeptide bonds, strategically placed so that pilins become inextensible

structures. Previously, we developed an isopeptide blocker, a short peptide that prevents the formation of the native isopeptide bond; however, the level of decoration is low. The aim of our work is to get an improved peptide that increases the level of decoration through rational and computational design.
Materials and Methods: We used two custom-made DNA constructs: the pFN18a expression vector, including the Spy0128 flanked by HaloTag and Avitag, and pBAD containing the differently designed peptide, expressed as a fusion protein with SNAP-Tag. Both pili protein and peptides were co-expressed and analyzed through SDS-PAGE and MT-based force spectroscopy.
Results: Although preliminary molecular docking assays suggested that the C96 peptide is a high-energy binding version of the isopeptide blocker, assay-based SDS-PAGE shows no decoration in C96. On the contrary, N14 and the D3 control peptide show a significant intervention of the isopeptide bond. On the other hand, MT experiments show that the C96, N14, and D3 peptides successfully prevent the formation of the isopeptide bond, rendering Spy0128 an extensible protein.
Discussion: In summary, our results suggest that computationally and rationally designed peptides are partially able to prevent the formation of the isopeptide bond in Spy0128. However, the most fundamental idea is that the size and physicochemical properties of the peptide need to be considered when designing it.
Acknowledgments: FONDECYT 1221064.

91. Unraveling wheat's response to sulfur starvation: analysis of TaSLIM1 homeologs as key regulators using integrated genomic and transcriptomic approaches. Ignacio Miño-Manzo (ignacio.mino@alumnos.uach.cl), Anita Arenas-M, Javier Canales. Universidad Austral de Chile, Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Valdivia, Chile.

Introduction: Sulfur deficiency significantly impacts plant growth and crop yield. In Arabidopsis, the transcription factor *SLIM1* (Sulfur LIMitation1) is a key regulator of sulfur response. Our RNA-seq analysis of sulfur-deprived bread wheat (*Triticum aestivum*) identified upregulation of *SLIM1* homeologs, particularly *TaSLIM1-5D* and *TaSLIM1-7D*, in both leaves and roots across developmental stages. This study aims to characterize *TaSLIM1-5D* and *TaSLIM1-7D* as functional orthologs of *AtSLIM1* and elucidate their roles in regulating the wheat response to sulfur deficiency.
Materials and Methods: We cloned *TaSLIM1-5D* and *TaSLIM1-7D* to generate transgenic Arabidopsis lines overexpressing these genes in a slim1_KO mutant background. We analyzed the presence of *SLIM1* binding motifs in the promoter regions of putative target genes (*TaLSU1*, *TaSDI*, *TaATPS*) of both transcription factors and examined their expression profiles by RT-qPCR in wheat tissues under sulfur-sufficient and deficient conditions across different growth stages.

Results: SLIM1 binding motifs were identified in the promoter regions of the putative targets of *TaSLIM1-5D* and *TaSLIM1-7D*. The expression of the transcription factors significantly increased under sulfur deficiency, showing strong correlation with the expression of the target genes. Transgenic Arabidopsis lines overexpressing *TaSLIM1-5D* and *TaSLIM1-7D* were successfully generated, providing a foundation for future functional complementation studies.
Discussion: Our findings strongly suggest that *TaSLIM1-5D* and *TaSLIM1-7D* are key regulators in wheat sulfur deficiency response, likely functioning as orthologs of *AtSLIM1*. This study provides crucial insights into sulfur homeostasis in wheat and lays the groundwork for improving sulfur use efficiency and enhancing crop productivity under sulfur-limited conditions.
Acknowledgment: This work was supported by FONDECYT N°1230833 and ANID—Millennium Science Initiative Program—ICN17-022.

92. eIF5 restricts ORFs expression based on a Kozak sequences stringency in *D. melanogaster.* Emiliano Molina[1] (emolinareyes@gmail.com), Carlos Oliva[2], Álvaro Glavic[1]. [1]Developmental Genetics laboratory, Faculty of Sciences, Universidad de Chile. [2]Faculty of Biological Sciences, Pontificia Universidad Católica de Chile.

Introduction: Translation is highly regulated by general factors (e.g. eIFs, eEFs, tRNA levels and mRNA levels) and structural features of mRNAs (e.g. 5' and 3' UTR, Kozak sequence and codon optimality).Here, we assessed whether there is a bias in ORF selection in transcripts with several ORFs and how this relates with their Kozak sequences (KS) and the availability of translation factors in different cells in *D. melanogaster.*
Material and Methods: We evaluated the expression of two translation reporters (WT and Mut). WT reporter contains a bicistronic transcript, where the first ORF has a weak KS and was fused to an HA epitope at the N-terminus, while the second has a strong KS and codes a C-terminal FLAG fused protein. In the Mut reporter the KS of the first ORF was mutated so that it was stronger than the second ORF. Using the Gal4/UAS system we expressed the reporters ubiquitously or in specific tissues and evaluated HA- and FLAG proteins levels by Western Blot and immunofluorescence (IF).
Results: Expression of the WT reporter in the whole animal, only produced the translation of second ORF (stronger KS), while the first ORF was not detected. This pattern was inverted using the Mut reporter. Changing the function of several initiation and elongation factors (eIFs and eEFs), revealed that knockdown of eIF5 was able to reverse ORF selection preference, allowing the translation from the weakest KS. Expression analysis of both reporters in various tissues by IF showed that ORF translation preference is not equivalent in the different tissues, besides these patterns were altered by modifying eIF5 levels.
Discussion: Together, these results suggests that eIF5 levels are important for ORF selection.
Acknowledgment: FONDECYT 1231105, FONDECYT 1231685

93. Biological activities of Chilean País grape pomace extracts: Polyphenol content, antioxidant capacity, cellular viability and migration on human dermal cells. Jessica Molina (jessica.molina@uss.cl)[1], Néstor Corro[1, 2], Paola G. Ojeda[2] and Carolina Añazco[1]. [1]Nutritional Biochemistry Laboratory. School of Nutrition and Dietetics. Faculty of Health Care Sciences. [2]Faculty of Medicine and Sciences. Universidad San Sebastián, sede Valdivia, Chile.

Introduction: The main type of solid waste in winemaking is grape pomace, mostly made of seeds and skins, rich in bioactive substances affecting biological processes. This study aimed to compare phenol and flavonoid content, and antioxidant activity of País grape pomace (PGP) from different years. Additionally, the impact of polyphenolic extracts on human skin cells was evaluated for potential use in cosmetics.
Materials and methods: PGP, composed by skins and seeds, was acquired by winery farmers of Central Chile (Cauquenes). Extracts were obtained using various strategies for recovering polyphenols. The total polyphenols content (TCP) was determined by Folin Ciocalteu method. The antioxidant capacity was determined through the ORAC test. The cell viability experiments and migration assays were done in the human dermal cell line CCD-1068sk.
Results: In the analysis of grape extracts from 2021 and 2022 in water, higher antioxidant capacity was found in the 2022 extract. The seed extract showed greater antioxidant activity compared to the total extract. The TPC was higher in the 2022 extract and seed extract, while the flavonoid content (TFCs) was similar in all samples. Aqueous polyphenolic extracts are used in *in vitro* studies to investigate their beneficial effects on human dermal cells. Different concentrations are tested to ensure no cytotoxicity and to observe modulation of migration properties.
Discussion: Aqueous extracts from Pais Grape pomace contain bioactive compounds suitable for use in cosmetic products.
Acknowledgment: FONDECYT 1212026 and FIC-R Maule BIP40027627-0.

94. Role of Cellular Prion Protein in the Development of Cardiac Hypertrophy in Neonatal Rat Cardiomyocytes. Evaristo Molina-Riquelme (evaristo.molina@ug.uchile.cl)[1,2], Úrsula Zúñiga-Cuevas[1,2], Leslye Venegas-Zamora[1,2], Valentina Parra[1,2,3]. [1]Laboratory for Cell Differentiation and Metabolism, Department of Biochemistry and Molecular Biology, Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile. [2]Advanced Center of Chronic Diseases (ACCDiS), Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile. [3]Systems Biology Center for the Study of Extremophile Communities from Mining Tailings (SYSTEMIX), O'Higgins University, Rancagua, Chile

Introduction: Pathological cardiac hypertrophy is a multifactorial condition that involves the

alteration of numerous processes within cardiomyocytes, ultimately leading to heart failure. Recently, cellular prion protein (PrP^c) has been identified as a potential novel biomarker for detecting maladaptive cardiac hypertrophy. Cardiomyocytes treated with norepinephrine and angiotensin showed increased hypertrophy markers as well as elevated levels of PrP^c. Additionally, overexpression of PrP^c reduced cell death induced by ischemia/reperfusion in mouse hearts, suggesting a cardioprotective role for this protein. Based on these findings, we hypothesized that PrP^c regulates the hypertrophic response in cardiomyocytes.
Materials and Methods: Neonatal rat ventricular myocytes (NRVMs) were treated with norepinephrine (NE) for 48 hours. The hypertrophic response was assessed by evaluating cell area and perimeter using immunofluorescence, as well as hypertrophy gene expression (ANP and BNP) and PrP^c expression by qPCR. Additionally, an siRNA targeting PrP^c was used to evaluate the role of the protein in the same hypertrophic parameters.
Results: Norepinephrine effectively induced cardiomyocyte hypertrophy, increasing cell area and perimeter. We also observed increased expression of hypertrophy markers such as ANP and BNP. Notably, PrP^c levels were elevated in cardiomyocytes treated with NE. To further investigate the role of PrP^c, we standardized the knockdown of this protein in our model.
Discussion: The increased levels of PrP^c in cardiomyocytes treated with NE suggest that this protein may play a role in the hypertrophic response. We are currently evaluating whether modulation of PrP^c levels alters the expression of hypertrophy markers. Collectively, this data highlights PrPc as a promising molecular target involved in cardiovascular diseases.
Funding: This project is funded by ANID FONDECYT 1230195 (VP), Anillo SYSTEMIX ACT210004 (VP), FONDAP 15130011 (VP); and Universidad de Chile grant Apoyo a la Infraestructura para la Investigación INFRA037/2023 (VP).

95. Fibroblast activated protein (FAP): a novel target for the early detection of cardiac fibrosis and fibrogenesis. Yanay Montano[1] (yanay.montano@gmail.com), Danica Jiménez-Gallegos[1], Ximena Calle-Chalco[1], Guerrero-Moncayo A[1], David Silva[1], Marcelo J Kogan[1], Sergio Lavandero[1,2]. [1]Advanced Center for Chronic Diseases (ACCDiS), Facultad de Ciencias Químicas y Farmacéuticas & Facultad Medicina, Universidad de Chile. [2]Cardiology Division, University of Texas Southwestern Medical Center, Dallas, Texas, USA.

Introduction: Cardiac fibrosis is a pathophysiological process characterized by excess extracellular matrix (ECM) deposition by cardiac fibroblasts (CFs). CF develops in myocardial infarction, hypertension, and different types of cardiomyopathies. There is no sensitive method for its diagnosis at an early stage of CF development. Fibroblast activation protein (FAP) is an interesting protein found in activated CFs.
Objective: To study FAP as a target for the early detection of cardiac fibrosis and fibrogenesis.

Methods: Primary neonatal rat CFs were treated with TGF-β1 10 ng/ml for 24, 48, and 72 h to induce their transformation to cardiac myofibroblasts (CMFs). The presence of FAP in activated CMFs was detected by Western blot assay, while FAP expression was assessed by indirect immunofluorescence. In addition, C57BL/6N mice were treated with Angiotensin II (Ang II) for 14 days (1,5 mg/kg/day), and immunohistochemistry for FAP was done. One-way ANOVA, post hoc Tukey, *p< 0.05, mean ± SD (n = 3-6). The bioethical protocol was the CICUA-CQyF2023-50 code.
Results: TGF-β1 (10 ng/ml at 72 h) induced the differentiation of CFs in CMF characterized by increases in expression of FAP and α-SMA genes, protein levels of collagen type I, fibronectin, and α-SMA and in cell area. Ang II stimulated the development of cardiac hypertrophy and hypertension among higher levels of FAP in the cardiac tissue.
Discussion: FAP can be used as a target for detecting cardiac fibrosis and fibrogenesis using a novel nanosystem that recognizes PAF.
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96. Identification of Cu resistance-associated genes and analysis of their synteny in *Pseudomonas* species isolated from Cauquenes copper tailing. Carlos Montiel Vera[1,2] (carlos.i.montiel.1998@gmail.com), Jaime Ortega[1,2], Mauricio Latorre[1,2,3]. [1]Laboratorio de Bioingeniería; Instituto de ciencias de la ingeniería; Universidad de O'Higgins, Rancagua, Chile. [2]Centro de biología de sistemas para el estudio de comunidades extremófilas de relaves mineros (SYSTEMIX), Universidad de O'Higgins, Rancagua, Chile. [3]Laboratorio de bioinformática y expresión génica, INTA, Universidad de Chile, Santiago, Chile.

Introduction: Copper (Cu) is an essential micronutrient existing in Cu^{1+} and Cu^{2+} forms, crucial for various biological processes. However, at high concentrations, it becomes toxic due to its ability to generate reactive oxygen species (ROS). Organisms have developed mechanisms to maintain copper homeostasis, avoiding toxicity while meeting nutritional needs. Bacteria use copper resistance systems like the CopA, CusABC and PcoAB to remove Cu from the cytoplasm. In Chile, mine tailings deposits, such as the Cauquenes tailings dam, contain high copper concentrations and host microorganisms, many of which belong to the genus *Pseudomonas*. This bacterial genus is of biotechnological interest due to its bioremediation capabilities and copper resistance. The study of genomic synteny around copper resistance genes in *Pseudomonas*, meaning the conservation of gene order, can offer new insights into their adaptation and survival in high-copper environments.
Materials and Methods: Ten bacterial isolates belonging to the genus *Pseudomonas* were sequenced and assembled, followed by a search for copper resistance genes in their genomes. Parallel growth assays were conducted at concentrations ranging from 1 mM to 20 mM $CuSO_4$, and survival was tested at 1 mM for 2 hours.
Results: Multiple copies of genes encoding copper resistance proteins were found in the bacterial genomes. Regarding growth, the bacteria could be classified into three groups

based on the MIC: 10 mM (low), 15 mM (medium), and 20 mM (high). High-resistance bacteria showed the highest number of efflux proteins (CopA and PcpAB) and elevated synteny between them.
Discussion: The presence of multiple copies of copper resistance genes suggests a robust genetic mechanism for coping with high copper levels. The correlation between high MIC values and synteny of copper efflux genes evidently a crucial role in bacterial copper resistance in *Pseudomonas* species.
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97. Cardioprotective role of Retro-enantio angiotensin-(1-8) in cardiomyocyte. Mora A[1], Calle, X[1]. Gina S[2], Kogan M[1], Lavandero S[1,2,3]. [1]Advanced Center for Chronic Diseases (ACCDiS), Facultad de Ciencias Químicas y Farmacéuticas y Facultad Medicina, Universidad de Chile. [2]ICBM, Facultad Medicina, Universidad de Chile. [3]Cardiology Division, University of Texas Southewestern Medical Center, Dallas, USA.
Acknowledgement: FONDAP 1523A0008.

98. Differential expression of chemokines in an *in-vitro* model of cisplatin-resistant gastric cancer. Yuselin Mora[1] (y.mora03@ufromail.cl), Kurt Buchegger[2,3,4], María Reyes[5], Barbara Mora[5], Carmen Ili[1, 2, 3], Tatiana Mellipan[1], Priscilla Brebi [1, 2, 3]. [1]Laboratory of Integrative Biology (LIBi), Center of Excellence in Translational Medicine (CEMT), Scientific and Technological Bioresource Nucleus (BIOREN), Universidad de La Frontera, Temuco 4780000, Chile. [2]Millennium Institute on Immunology and Immunotherapy, Santiago, Chile. [3]BMRC, Biomedical research consortium-Chile. [4] Department of Basic Sciences, School of Medicine, Universidad de La Frontera, Temuco, Chile. [5] Instituto de Ciencias Biomédicas, Facultad de Ciencias de la Salud, Universidad Autónoma de Chile, Temuco, Chile.

Introduction: Gastric cancer (GC) is the third deadliest and fifth most common cancer globally. In Chile is the second most common cancer, with a high mortality rate in the Araucanía region, constituting a significant public health issue. The main obstacles for curing GC are late diagnosis and the development of chemoresistant cells to the widely used drug cisplatin. The mechanism of this resistance has not been fully elucidated. However, it has been associated with the secretion of inflammatory mediators such as cytokines and chemokines. Therefore, the detection of these soluble inflammatory factors in resistant phenotypes may contribute to understanding the mechanisms underlying cisplatin resistance and determine potential therapeutic targets to improve treatment response in GC patients. This study aims to identify the expression of human chemokines in sensitive cisplatin-resistant gastric cancer cell lines.
Materials and Methods: 38 human chemokines were evaluated using an antibody microarray from cell culture supernatants of AGS-WT, AGS-RCDDP, SNU 601, SNU 638 lines, RPMI 1640 medium was used as a control. The chemokine microarray images were

analyzed using Image J software.
Results: Chemokine expression profiles, as determined by microarray analysis, varied significantly among the gastric cancer cell lines. While AGS-WT expressed a wide spectrum of chemokines (GRO, GROα, CXCL16, and IL-8), AGS-RCDDP exhibited reduced expression of CXCL16. Additionally, SNU-601 expressed CXCL12/SDF-1α and IL-8, whereas SNU-638 expressed only IL-8. These results suggest that cisplatin resistance is associated with alterations in chemokine expression profiles, characterized by a decrease in CXCL16 and CXCL12/SDF-1α, and an increase in IL-8.
Discussion: CXCL16 and IL-8 play key roles in gastric cancer, affecting tumor growth and treatment resistance. IL-8, promoting inflammation and angiogenesis, is a promising therapeutic target. More research is needed to confirm these findings and understand the underlying mechanisms.
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99. Genetic identification of *hydroperoxide lyase* (HPL) and its relationship in the aroma pathway of *Fragaria x ananassa fruits*. Felipe Moraga-Maldonado[1] (felipe.moraga290999@gmail.com); Francisca Rodríguez-Arriaza[1]; Francisca Hormazabal-Abarza[1]; Patricio Ramos[2]; Luis Morales-Quintana[1]. [1]Multidisciplinary Agroindustry Research Laboratory, Institute of Biomedical Sciences, Faculty of Health Sciences, Autonomous University of Chile, Talca, Chile. [2] Plant Microorganism Interaction Laboratory, Instituto de Ciencias Biológicas, Universidad de Talca, Talca, Chile.

Introduction: The commercial strawberry (*Fragaria x ananassa*) is a horticultural crop of great importance due to the flavor and aroma of its fruit. The aroma forms an important part of the composition of the fruit, which is synthesized by a series of chemical and enzymatic reactions that generate volatile compounds. Hydroperoxide lyase (HPL) is an enzyme involved in aroma formation by breaking down unsaturated fatty acid hydroperoxides from linoleic and linolenic acids to form volatile aldehydes and various oxygen-containing acids. To date, there is no complete description of the biosynthesis pathway of volatile compounds associated with aroma in strawberries and, therefore, there is no information regarding the presence or function of the HPL enzyme in this fruit. For this reason, the objective of this research is to identify and analyze the presence of the enzyme and genes coding for HPL in strawberries at different stages of ripening.
Methods: “Camarosa” strawberries were used at different stages of ripening. In addition, strawberries treated with abscisic acid (ABA) and auxins (AUX) were used and evaluated at different times (0, 24, 48 hours) post-treatment, and then the expression of *FaHPL* genes was measured using molecular techniques.
Results: Five isoforms were found in the strawberry genome that identified the expression of *HPL*. The presence of HPL was observed in all stages of ripening in different degrees of

expression, although the highest levels were found in small green and large green stages. In addition, there were significant variations in the expression of FaHPL genes after treatment with ABA and Auxin hormones, showing the importance of these hormones in the modulation of the precursors of important volatile compounds in strawberry fruits.
Acknowledgements: Fondecyt regular 1220782 and ANILLO ATE220014.

100. Protective effect of SNP rs2043556:T>C on breast tumorigenesis processes. Sarai Morales-González[1] (sarai.morales@ug.uchile.cl), Julio C. Tapia[2], Lilian Jara[1]. [1]Programa de Genética Humana, ICBM, Facultad de Medicina, Universidad de Chile. [2]Programa de Biología Celular y Molecular, ICBM, Facultad de Medicina, Universidad de Chile.

Introduction: Breast cancer (BC) is the most common cancer in women worldwide, with 2.4 million new cases in 2022. In Chile, BC is the leading cause of cancer death in women, with a rate of 18.2/100,000. MicroRNAs (miRNAs) have been proposed as susceptibility genes for BC, that regulate cellular processes such as differentiation, proliferation, and apoptosis. Single nucleotide polymorphisms (SNPs) are the most common type of variant in the human genome. SNPs in miRNA genes can influence cancer susceptibility, including BC. The SNP rs2043556:T>C is located in the precursor region of miRNA-605, which has been reported to inhibit the expression of important oncogenes, such as MDM2, a critical inhibitor of p53. Previously, our group showed that SNP rs2043556:T>C was significantly associated with decreased BC risk. Thus, the objective of this study was to evaluate *in vitro* the impact of SNP rs2043556:T>C on the breast tumorigenesis process using BC cell lines.
Materials and Methods: BC cell lines MCF-7 (luminal A, less-aggressive) and MDA-MB-231 (triple-negative, highly-aggressive) were transfected with pre-miR-605-T, pre-miR-605-C, or empty vector. Subsequently, levels of mature miRNA-605 expression, viability, apoptosis, migration, and cell invasion were determined.
Results: The results indicate that the rs2043556-C allele affects the expression of mature miRNAs-605 depending on the cell line type. The rs2043556-C allele decrease cell viability in luminal A and triple-netive cell lines. Additionally, rs2043556-C allele increases apoptosis in MCF-7 cells but decreases it in MDA-MB-231 cells. Cell migration and invasion decreases when BC cells are transfected with the SNP rs2043556-C allele.
Discussion: The C allele of rs2043556 may have a protective effect on the luminal A and triple-negative BC. This SNP could be considered as a biomarker for BC.
Acknowledgment: FONDECYTs 1200049 (LJ) & 1220353 (JCT); ANID/PhD Scholarship 21210763 (SM-G).

101. Conserved NPF6.3 unique features evolved alongside Mesangiospermae and are critical for its transceptor function. Jonathan A. Morales (jon.moralese@gmail.com), Eleodoro Riveras, Rodrigo A. Gutiérrez. Millennium Institute for Integrative Biology (iBio).

Millennium Institute Center for Genome Regulation (CRG). Institute of Ecology and Biodiversity (IEB). Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

Introduction. NPF6.3 is a nitrate transceptor that plays a role in both nitrate transport and nitrate sensing. NPF6.3 functions as a unique dual-affinity nitrate transporter, unlike other nitrate transporters. NPF6.3 participates in both high- and low-affinity nitrate transport systems. NPF6.3 constitutes the first layer of nitrate perception. The structural basis making it unique among other transporters and receptors remains unveiled. To understand the structural basis of the unique characteristics of NPF6.3, we developed a novel method to identify differentially conserved residues within groups of proteins.
Methods. To identify differentially conserved residues, we contrasted the conservation patterns in NPF6.3 orthologs and paralogs. First, (i) we selected relevant orthologs and paralogs of AtNPF6.3 and (ii) performed multiple sequence alignments (MSA). Then, (iii) we quantified three different conservation scores and (iv) calculated (iv) delta conservation scores (ΔS). Finally, we (v) identified differentially conserved residues by integrating ΔS information across the MSAs.
Results. We found 17 differentially conserved residues clustered in two regions of NPF6.3, which we labeled DCR1 and DCR2. DCR1 is in the largest intracellular loop, while DCR2 is located at the C-terminal region of the transporter. Our methodology revealed that DCRs were exclusively found in Mesangiospermae species.
Discussion. Our novel methodology identified differentially conserved residues and highlighted potential functional features of NPF6.3 that are conserved across evolutionarily distant species.
Acknowledgment. Fondecyt 1220594 and 11230913. Millennium Institute of Integrative Biology (iBio), ICN17_022. Center for Genome Regulation is a Millennium Institute Project ICN2021_044.

102. Abscisic acid application for sustainable agriculture under climatic change conditions focus on strawberry fruit production. Ricardo I. Castro[1], Daniel Bustos[2], Carolina Parra-Palma[3], Sebastian Flores[3,4], Patricio Ramos[4], Luis Morales-Quintana[3]. [1] Multidiciplinary Agroindustry Research Laboratory, Instituto de Ciencias Aplicadas, Facultad de Arquitectura, Construcción y Medio Ambiente, Universidad Autónoma de Chile. [2] Laboratorio de Bioinformática y Química Computacional, Departamento de Medicina Traslacional, Facultad de Medicina, Universidad Católica del Maule, Talca, Chile. [3] Multidiciplinary Agroindustry Research Laboratory, Instituto de Ciencias Biomédicas, Facultad de Ciencias de la Salud, Universidad Autónoma de Chile. [4] Plant-Microorganism Interaction Laboratory (PMIL), Instituto de Ciencias Biológicas, Universidad de Talca.

Introduction: Global water scarcity is a critical issue that leads to significant crop losses, worsened by increasing desertification. Chile, classified as highly vulnerable by the IPCC,

experiences pronounced aridity in its central and southern regions due to climate change. This situation poses severe threats to agriculture and food security.
Methodology: To address water deficits, the application of the hormone abscisic acid (ABA) has been explored. ABA, naturally produced by plants under water stress, enhances phenolic compounds and anthocyanins, thereby improving antioxidant capacity and aiding plant adaptation. In this study, ABA was encapsulated in a controlled-release form and applied to strawberry crops. The experimental setup involved a 50% reduction in irrigation, with evaluations of various nutritional and physiological parameters, including size, weight, color, and volatile compound composition.
Results: The controlled-release application of ABA successfully maintained strawberry production under reduced irrigation conditions. The treatment resulted in increased phenolic compounds and anthocyanins, enhancing antioxidant capacity. Additionally, changes in volatile esters, reduced lipid peroxidation, modulated antioxidant enzymatic activity, and increased proline content were observed in the strawberry fruits.
Conclusion: Encapsulated ABA in controlled-release forms offers a promising approach to enhancing crop resilience under changing climate conditions and water scarcity. This technique helps maintain essential nutritional and physiological parameters, providing a viable solution for sustainable agriculture.
Funding: Projects: FONDECYT #1220782, #1211057 and #1240771, FONDECYT PostDoctoral #3240463, and Anillo #ATE220014.

103. DRP1 and Bcl-xL interaction in therapy-induced senescence progression in a model of colorectal cancer. Pablo Morgado-Cáceres[1,2,3,5], Ulises Ahumada-Castro[1], Eduardo Silva-Pavez[1], Andrea Puebla-Huerta[1], Osmán Díaz-Rivera[1], Valentina Parra[2,3,4], César Cárdenas[1,5]. [1] Center for Integrative Biology, Faculty of Sciences, Universidad Mayor, Santiago, Chile. [2] Laboratory for Cell Differentiation and Metabolism, Department of Biochemistry and Molecular Biology, Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile. [3]Advanced Center of Chronic Diseases (ACCDiS), Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile. [4]Systems Biology Center for the Study of Extremophile Communities from Mining Tailings (SYSTEMIX), O'Higgins University, Rancagua, Chile. [5]Geroscience Center for Brain Health and Metabolism.

Introduction: Colorectal cancer (CRC) is one of the leading causes of death. Chemotherapy treatments typically promote CRC cell death; however, some cells can enter a state known as therapy-induced senescence (TIS). This form of cellular senescence involves a stable cell cycle arrest, accompanied by a unique secretome and other distinctive features. In various cancers, low levels of DRP1, a mitochondrial fission protein, have been linked to increased susceptibility to chemotherapeutics, suggesting that reduced mitochondrial fission mediates this effect, although a scientific consensus is lacking. Interestingly, DRP1 is known to interact directly with the anti-apoptotic protein Bcl-xL, which

is highly expressed in different models of senescence. Therefore, we hypothesize that DRP1 interacts with Bcl-xL during TIS, promoting the establishment of senescence rather than cell death.
Materials and Methods: We used the HCT-116 colorectal cancer cell line, including wild type, DRP1 knockout, and MFF knockout variants. To induce TIS, cells were treated with 62.5 µM Doxorubicin (Doxo) for 2 days. Survival and senescence were assessed via crystal violet staining, flow cytometry, SA-β-galactosidase activity, and Western blot analysis of senescence-related proteins. We also evaluated the effects of the pharmacological inhibition of DRP1 and Bcl-xL. A proximity ligation assay (PLA) was used to study DRP1/Bcl-xL interaction.
Results: Doxo treatment efficiently induced therapy-induced senescence (TIS) in HCT-116 cells. The absence of DRP1, but not its pharmacological inhibition, promoted cell death during this process. Interestingly, the absence of MFF, another protein related to mitochondrial fission, did not lead to cell death. Additionally, a strong DRP1/Bcl-xL interaction was observed in cells three days after TIS induction.
Conclusions: DRP1 appears necessary for CRC cell survival during TIS by mitochondrial fission-independent mechanisms. This is the first report on DRP1/Bcl-xL interplay in TIS. Further analyses are required to understand the importance of this interaction.
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104. Gene regulatory network analysis to uncover conserved plant drought stress responses. Tomas C Moyano [1,2](tcmoyanoyugovic@gmail.com), J. Sebastian Contreras-Riquelme[1,2], Nathan R. Johnson[1,2,3], Nicolas Zapata, Adrian Moreno, Jose M Alvarez[1,2]. [1]Centro de Biotecnología Vegetal, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago, Chile. [2] Millennium Science Initiative – Millennium Institute for Integrative Biology (iBio), Santiago, Chile. [3] Centro de Genómica y Bioinformática, Facultad de Ciencias, Universidad Mayor, Santiago, Chile.

Introduction: One of the main problems for plant life is the availability of water. Drought events have become more frequent because of climate change and are greatly devastating to agricultural productivity. Drought impacts the transcription of thousands of genes due to complex drought-responsive gene regulatory networks (GRNs). Although some plants are more tolerant than others under drought, limited information is available to assess variation and what is conserved in their GRNs. The current study aims to identify the conserved components of the GRNs across diverse model and commercially important plants, like Arabidopsis, tomato, rice, and maize, utilizing the most recent transcriptomic data associated with drought conditions and data from chromatin accessibility studies.

Materials and Methods: We organized, filtered, and analyzed metadata profiles related to drought treatment. Differentially expressed genes (DEGs) were derived for each experiment and further filtered to identify shared responses among the plants. Furthermore, we predicted GRNs from chromatin accessibility and gene expression profiles. We compared drought stress-related genes among species to detect common and divergent patterns using Orthofinder.
Results: We found a group of consistently drought-induced genes in Arabidopsis, tomato, rice, and maize. Our analysis provided evidence of a conserved transcriptional circuit underlying their drought responses through the identification of key transcription factors and regulatory components consistently involved in such responses. The GRNs constructed from our data illustrate the complex networks of interactions that control gene expression under drought stress.
Discussion: We provided a snapshot of the transcriptional networks controlling drought responses in plants. Identifying convergent and divergent elements in these GRNs offers insights into drought tolerance mechanisms. This approach establishes a reference for each species, aiding in hypothesisformulation about genes and regulatory components necessary for drought adaptation. Understanding these networks will support breeding programs and genetic engineering efforts to improve drought tolerance in crops.
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105. Targeting the MALAT1 long noncoding RNA with an LNA-modified Deoxyribozyme. Marcelo Muñoz[1] (marcelomunoz.gon@gmail.com), Adrián A. Moreno[2], Marjorie Cepeda-Plaza[3], Rodrigo Aguilar[1]. [1]Institute of Biomedical Sciences. [2]Centro de Biotecnologia Vegetal. [3]Chemical Sciences Department, Universidad Andres Bello, Santiago, Chile.

Introduction: Given the increasing roles that noncoding RNAs are playing in human disease, they are promising targets for loss-of-function and pharmacological strategies. Here we report the design and use of Deoxyribozymes (or DNAzymes), which are artificial single-stranded DNA oligonucleotides with autonomous catalytic activity that can be designed to recognize and cleave any target RNA. With a specific type of DNAzyme (named 10-23), we aimed to specifically degrade the long noncoding RNA MALAT1, one of the most overexpressed transcripts in human cancer cells.
Materials and Methods: *In vitro* RNA-cleaving assays were performed at 37ºC against a 20-nt segment of MALAT1 in single-turnover conditions (DNAzyme:RNA = 1:10). We used different concentrations of the required divalent metal cofactor, Mg^{2+} and Ca^{2+}. On the other hand, an *in vivo* analysis was carried out in cancer cells where the LNAzymes were transfected using Lipofectamine 3000.
Results: The reaction rate constant (kobs) for a non-modified DNAzyme ranged from 0.02 to 0.8 min^{-1}, at 2 mM and 10 mM metal concentration, respectively. When DNAzyme

nucleotides were modified with locked nucleic acids (LNA) modifications at the 3' and 5' end, kobs values increased, ranging from 0.15 to 1.5 min^{-1}. Finally, we incubated human cells with LNA-modified Deoxyribozymes, finding median effective concentrations for RNA degradation (EC50) between 125 and 250 nM. Thus, we offer an alternative approach for loss-of-function experiments centered on RNAs.
Discussion: This autonomous silencing system can be a great tool for gene regulation, demonstrated by its high rate-constant in *in vitro* assays and its effectiveness in cell assays.
Acknowledgment: UNAB DI-05-24-REG, FONDECYT 1240853.

106. Evolutionary, structural, and kinetic insights into methanogenic archaea's pyruvate/phosphoenolpyruvate node enzyme regulation. Sebastián M. Muñoz (Sebastian.munoz.m@ug.uchile.cl), Ignacio Aravena-Valenzuela, Antonia Alarcón-Saavedra, Felipe González-Ordenes, Nicolás Fuentes-Ugarte, Gonzalo Quiñones-Pérez, Víctor Castro-Fernández, Victoria Guixé. Laboratorio de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Chile. Santiago, Chile.
Methanogenic archaea cannot thrive using complex sugars as their sole carbon source. However, these microorganisms accumulate glycogen, which is used as an energy source when substrates for methanogenesis are depleted. The switch from gluconeogenic to glycolytic metabolism involves a regulatory mechanism, mainly in the enzymes that catalyze irreversible reactions, such as those of the pyruvate/phosphoenolpyruvate node, which are pyruvate kinase (PK) and phosphoenolpyruvate synthase (PEPS). Although the PK enzymes are homologous across the three domains of life, they are regulated by several allosteric effectors in different phylogenetic groups, such as fructose-1,6-bisphosphate in eukaryotes and bacteria. In contrast, AMP regulates the Pk activity in some bacteria and methanogenic archaea. Furthermore, it is currently unknown if the PEPS enzyme from methanogenic archaea is allosterically regulated.
We used an ancestral sequence reconstruction approach to investigate the evolution of AMP specificity at the allosteric site of PK enzymes in methanogenic archaea. We constructed a phylogenetic tree with 1,063 PK sequences and the most probable sequences of a common ancestor of PKs from all three domains of life (AncCPK) and the last common ancestor of *Methanococcales* order of methanogenic archaea (AncMcPK) was inferred. We recombinantly expressed and purified both ancestral PKs and determined their kinetic parameters. We also assessed their regulation by several effectors, revealing that AMP does not activate either enzyme, suggesting AMP activation is a more recent trait in the PKs family. However, we observed significant inhibition by the NaPPi effector. Additionally, we recombinantly expressed and purified the PEPS enzyme from *Methanocaldococcus maripaludis* (MmPEPS). The enzyme is an oligomer of 1.7 MDa as determined by SEC-MALS. For AncCPK, we determined the X-ray structure at 3.4 Å resolution and established preliminary conditions for determining the 3D structures of AncMcPK and MmPEPS via cryo-EM.
Funding: FONDECYT 1231263 and 1221667.

107. Transcriptional rewiring of master transcription factors for response optimization. Macarena Muñoz[1,2](macarena.amsilva@gmail.com), Ariel Cerda[1,2], Jonatan Morales[2], Eleodoro Riveras[2], Rodrigo Gutiérrez[2,3], Luis Larrondo[2], José M. Álvarez[1,2]. [1]Centro de Biotecnología Vegetal, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago, Chile. [2]Agencia Nacional de Investigación y Desarrollo – Millenium Science Initiative Program, Millenium Institute for Integrative Biology (iBio), Santiago, Chile. [3]Center for Genome Regulation (CRG), Institute of Ecology and Biodiversity (IEB), Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile

Introduction: Nitrogen (N) is a limiting factor for plant development that induces changes in gene expression orchestrated by master transcription factors (TFs). However, excessive use of N-containing fertilizers is a significant environmental challenge. Thus, there is a pressing need to enhance nitrogen use efficiency in plants. Within the nitrogen pathway, NLP7 and TGA1 are TFs that show complementary effects. Their independent overexpression enhances plant growth, nitrogen uptake and assimilation in *Arabidopsis thaliana*, however regulation between them remains unknown. Through synthetic biology, it is possible to rewire regulatory circuits and cellular functions. This involves combining genetic tools and engineering principles to assess the impact of directly connecting TFs that do not usually interact. Our aim is to connect NLP7 and TGA1 and their regulatory contributions to the nitrogen pathway by transcriptional rewiring to potentiate the N-mediated gene expression changes and optimize nitrogen use efficiency.
Materials and methods: We integrated extensive genomic data on TF-binding and TF-regulation for NLP7 and TGA1 to identify shared and unique pathways. Gene lists intersection analysis was performed through Genesect tool, to elucidate the TFs regulatory interactions and downstream targets for gene circuits outputs. Plasmids with synthetic promoters and TFs were built through Golden Gate assembly and transfected into *Arabidopsis thaliana* leaves protoplasts from Col-0, *nlp7* and *tga1 tga4* plants, treated with 10mM KCl or KNO_3 to assess transcriptional outputs by dual luciferase assays.
Results: We found a high proportion of unique gene responses and biological functions controlled by each TF, encouraging the transcriptional rewiring.
We measured the synthetic promoter's transactivation through luciferase signal for all genotypes. We observed there was at least a 2 times higher activation of the synthetic promoters if they included its cognate TF in the same plasmid for Col-0 protoplasts. This difference was even higher in *nlp7* (up to 20 times) and *tga1 tga4* (up to 3 times) protoplasts, showing influence of endogenous TFs on the synthetic promoters.
Discussion: Through bioinformatic analysis and high-throughput plasmid assembly, it is possible to evaluate and characterize different gene circuits and regulatory elements to understand and further optimize the nitrogen response and use efficiency.
Acknowledgement: FONDECYT Regular 1210389, Instituto Milenio de Biología Integrativa iBio Chile ICN17_002.

108. Cardiomyocyte vascular cell adhesion molecule 1 (VCAM-1) increases during palmitic acid-induced cardiomyocyte hypertrophy and cell death. Laura Navarrete-Gallegos[1](laura.navarrete@ug.uchile.cl), Elsa Rocío Bascuñán[1], Danica Jimenez-Gallegos[1], Sebastián Urquiza[1], Claudia Muñoz[1], Jaime A Riquelme[1], Magda C. Díaz-Vesga[1], Fernanda Zapata-Neweu[1], Brenda Becerra-Leiva[1], Mario Chiong[1], Marioly Müller[1,2], Mayarling F Troncoso[1,2], Sergio Lavandero[1,3]. [1]Advanced Center for Chronic Diseases (ACCDiS), Faculty of Chemical and Pharmaceutical Science & Faculty of Medicine, University of Chile, Santiago, Chile. [2]Department of Medical Technology, Faculty of Medicine, University of Chile. Santiago, [3]Cardiology Division, University of Texas Southwestern Medical Center, Dallas, USA.

Introduction: Cardiovascular diseases are one of the main causes of death globally and obesity is a key cardiovascular risk factor. High plasmatic concentration of palmitic acid has been detected in high-fat diet-induce obesity where cardiomyocyte hypertrophy and death could contribute to obesity-induced cardiomyopathy. We have established that the Vascular Cell Adhesion Molecule 1 (VCAM-1) is expressed in cardiomyocytes and its specific deletion affects heart structure and function in VCAM-1-knockout mice fed with high fat diet or exposed to ischemia/reperfusion (unpublished data). However, the role of VCAM-1 in cardiomyocytes exposed to palmitic acid reminds unknown. Our aim was to evaluate the expression of cardiomyocyte VCAM-1 in palmitic acid-induced hypertrophy and cell death.
Materials and Methods: Neonatal rat cardiomyocytes were treated with increasing palmitic acid (PA) concentrations (6.25, 12.5 and 25 nM) for 10, 24 and 48 h (Bioethical protocol CQyF2024-60, U. Chile). Cell hypertrophy was assessed by expression of specific biomarkers (RT-qPCR and immunoblot: ANP and beta-MHC), cell area and perimeter. Cell death was evaluated by propidium iodine/Yo-pro nuclei staining and expression of cleaved caspase 3. VCAM-1 mRNA and protein expression were also evaluated. Data were expressed as mean ± SD and the statistical analysis were two tailed *t* test and one- or two-way ANOVA followed by Turkey's *post hoc*. $p<0.05$ was considered significant.
Results: Increasing PA concentration and exposure time led to cardiomyocyte hypertrophy and subsequent cell death. Specifically, high PA concentration (25 nM) for 48 h significantly increased cardiomyocyte death compared with control. In response to low PA concentration (6.25 nM) increased cardiomyocyte VCAM-1 expression was observed in parallel to the development of cardiomyocyte hypertrophy but not with cell death.
Discussion: These preliminary results suggest that cardiomyocyte VCAM-1 expression increased in response to PA cytotoxicity, leading to cardiomyocyte hypertrophy and prevention of cell death.
Acknowledgment: FONDECYT 1240443, FONDAP 15130011-1523A0008, FONDECYT Postdoctorado 3240492.

109. Regulation of SALL2 by the Wnt/b-catenin pathway activator Tankyrase. Benjamin Neira (beneira2020@udec.cl), Paula Medina, Aracelly Quiroz, Ariel Castro, Roxana Pincheira. Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Chile.

Introduction: SALL2 is a member of the SALL family of transcription factors involved in neurogenesis, neuronal differentiation, and cancer. In the context of colorectal cancer (CRC), SALL2 expression is decreased. We have evidence that SALL2 regulates AXIN2, a negative regulator of the Wnt/β-catenin pathway; consistently, loss of SALL2 in human CRC tissues correlates with increased nuclear β-catenin, a marker of Wnt pathway activation and cancer progression. Why SALL2 decreases in CRC is currently unknown. Preliminary work indicates that XAV939, a Wnt pathway inhibitor that acts by inhibiting Tankyrase (TNKS), increases the expression of SALL2. Tankyrases interact with target proteins and regulate their interactions and stability through poly ADP-ribosylation. Here, we investigate the potential regulation of SALL2 by Tankyrases.
Materials and Methods: We use bioinformatics to search for conserved TNKS binding sites in the SALL2 protein sequence. Cell fractionation and immunocytochemistry evaluated the subcellular location of TNKS and SALL2 in HEK293T and SW480 cells. Western Blot evaluated changes of SALL2 protein upon overexpression of FLAG-TNKS1 and FLAG-TNKS2. For the co-immunoprecipitation experiment, HEK293T cells were transfected with FLAG-TNKS1 plasmid and immunoprecipitated with an anti-FLAG antibody.
Results: Our study showed that SALL2 contains a conserved putative TNKS binding site at position 786. We found that SALL2 and TNKSs share subcellular location at the nucleus. Furthermore, compared to the control condition, a significant decrease in SALL2 levels occurs upon transfection of TNKS1, TNKS2, or both. Lastly, SALL2 coimmunoprecipitated with TNKS 1-FLAG, confirming their interaction.
Discussion: Our findings suggest that TNKS1 and TNKS2 co-localize and interact with SALL2, indicating that these enzymes could negatively regulate the stability of SALL2. However, further studies are needed to understand this interaction fully.
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110. A novel script for semi automatic sequence alignments finds a set of endomembrane trafficking genes likely involved in drought tolerance in eucalyptus. Gabriel Neira-Valenzuela[1,2](gabriel.neiravalenzuela@gmail.com), Y. Stappung[1,3], R. Herrera[1,3], Lorena Norambuena[1,2]. [1]Multidisciplinary Center for Biotechnology and Molecular Biology for Climate Change Adaptation in Forest Resources (CeBioCliF), [2]Plant Molecular Biology Centre, Department of Biology and Faculty of Science, Universidad de Chile. [3]Biological Science Institute, Universidad de Talca.

Introduction: One of the consequences of climate change is the drought predicted to several areas of the planet. Given this, it is necessary to develop knowledge and

technologies that allow us to adapt to the future context, and adapt the resources we use, such as plants. For model organisms, there is a lot of data that has been collected regarding molecular players that contribute to drought tolerance. However, for forest trees the information is scarce. It is of our interest to identify molecular players involved in endomembrane trafficking and drought tolerance in the productive important species such eucalyptus. Comparative genomic approaches allow the identification of molecular players in the organism of interest based on the information of model organisms. Subsequently, functional characterization of such factors would relate them to molecular, cellular and/or physiological processes. Comparative genomics imply to work with a large pool of information looking for generalities that will help us to select a smaller and more specific set of data, but data collection and sequence analysis of large data sets can be problematic and time consuming.
Materials and Methods: We have developed a script that performs a semi-automated alignment of a large number of sequences using the application programming interface (API) and the Bio.Blast Package libraries based on python.
Results: Eucalyptus genes likely involved in endomembrane trafficking and related to drought tolerance were selected as candidates for further molecular and functional characterization. The alignment of protein sequences and identification of functional domains of those candidates with genes identified. Moreover, the alignment of protein sequences and identification of functional domains of those candidates was performed to validate the molecular identity.
Discussion: Our results confirm that the developed script is a powerful tool for identification of molecular players in eucalyptus.
Acknowledgment: CeBioCliF ATE220043

111. Cardiometabolic effect of angiotensin-(1-9) on diabetic cardiomyopathy. Ignacio Norambuena-Soto[1,2] (inorambuenasoto@coh.org), Marcelo Kogan[1], Mario Chiong[1], Zhao V. Wang[2,4,5], Sergio Lavandero[1,3]. [1]Advanced Center for Chronic Diseases (ACCDiS), Facultad Ciencias Químicas y Farmacéuticas & Facultad Medicina, Universidad de Chile, Santiago, Chile. [2]Department of Diabetes and Cancer Metabolism, Beckman Research Institute, City of Hope National Medical Center, Duarte, California, USA [3]Cardiology Division, University of Texas Southwestern Medical Center, Dallas, Texas, USA. [4]Irell and Manella Graduate School of Biological Sciences, City of Hope National Medical Center, Duarte, California, USA. [5]City of Hope Comprehensive Cancer Center, Duarte, California, USA.

Introduction: Diabetic cardiomyopathy is a clinical condition of ventricular dysfunction that develops in type 2 diabetes mellitus, obese and insulin resistance patients. The heart under this metabolically challenged condition manifests accumulation of free fatty acids, such as palmitate, and lipotoxic stress, which decreases insulin sensitivity and adversely affects the activity of proteins responsible for glucose transport, such as Akt. Angiotensin-(1-9) is a novel peptide of the counter-regulatory axis of the renin-angiotensin system with

cardioprotective effects, which reduces myocardial infarct size, blood pressure, and inflammation. However, it remains unknown whether angiotensin-(1-9) modulates insulin sensitivity in the heart and cardiomyocytes in the context of lipotoxic stress.
Objective: To investigate the cardiometabolic effects of angiotensin-(1-9) on diabetic cardiomyopathy.
Methods: Lipotoxic stress *in vivo* was induced by feeding mice with high-fat diet, followed by angiotensin-(1-9) administration for four weeks using osmotic mini-pumps. Cardiac function was assessed by echocardiography, and insulin sensitivity was assessed after a bolus injection of insulin. Lipotoxic stress *in vitro* was induced by high glucose medium plus palmitate in primary cardiomyocytes. *In vitro,* insulin sensitivity was assessed after treating cardiomyocytes for 10 min with insulin. All animal procedures were approved by both the institutional ethics review committee of Universidad de Chile (code: 21444-CQF-UCH) and the IACUC of City of Hope (animal protocol: #21070).
Results: In HFD-fed mice, Ang-(1-9), after four weeks of treatment, improved myocardial function and insulin sensitivity in the heart and skeletal muscle but showed no effects in adipose tissue or liver. These results suggest that Ang-(1-9) has a tissue-specific impact, preferentially acting on muscle. Angiotensin-(1-9) also increased the insulin signaling under lipotoxic stress through the AT2 receptor.
Conclusion: Angiotensin-(1-9) improves insulin signaling in cardiomyocytes and the heart under lipotoxic stress.
Funding: FONDECYT postdoctoral 3210496 (INS), FONDAP 1523A0008, FONDECYT 1240443 (SL), AHA 19IPLOI34760325 and ADA 7-20-IBS-218 (ZVW).

112. Potential antiviral activity of a botanical extract against Human Immunodeficiency Virus Type 1 (HIV-1).
Gustavo Salas[1] (g.salastapia@uandresbello.edu), Jorge Rivas[2], Loretto Contreras-Porcia[2], Francisca C Bronfman[1], Gloria Arriagada[1]. [1]Instituto de Ciencias Biomédicas, Facultad de Medicina; [2]Departamento de Ecología y Biodiversidad, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago, Chile.

Introduction: Human Immunodeficiency Virus Type 1 (HIV-1) is the causative agent of the acquired immunodeficiency syndrome. Despite new pharmacological treatments HIV-1 continues to spread worldwide, with drug resistance arising slowly. With the need of new antiviral options botanical extracts are attractive candidates. They have been reported to have effects against viruses such as HSV, DENV or SARS-CoV-2. In this study we assay *in vitro* the potential effects of a botanical extract against HIV-1.
Materials and Methods: Cytotoxicity of the botanical extract was evaluated by MTT in HEK293T cells. Single-round infection HIV-1-luciferase (HIV-Luc) were generated by co-transfection of HEK293T cells with pNL43Δenv.luc and pMDG (VSV-envelope) or pHIT456 (Amphotropic-MLV-envelope). Antiviral activity was assayed using non-lethal doses of the

botanical extract and challenging HEK29T cells with VSV-HIV-luc or Ampho-HIV-luc. Luciferase activity was evaluated 24h post-infection.
Results: Cytotoxicity assays showed that cells were viable up to 100 µg/mL of the botanical extract. When cells were challenged with VSV-HIV-luc and treated with different doses of the botanical extract (5 to 80µg/mL), we observed up to 40% reduction of the reporter activity compared to vehicle. Ampho-HIV-Luc reporter activity was not inhibited after treatment with the same doses of the extract.
Discussion: Our results suggest that the botanical extract can inhibit HIV-1 reporter activity only when the entry mechanism is endocytosis. To verify this, we are currently testing the botanical extract in human monocytic cells infected with the HIV-luc with its native envelope (plasma membrane fusion) or VSV-G (endocytosis).
Disclosure: The composition and origin of the botanical extract is being analyzed for potential technological IP protection.

113. Use of Cu-resistant bacteria with plant growth-promoting traits from Cauquenes tailings for promoting *in-vitro* tomato growth. Jaime Ortega[1,2,3] (jaime.ortega@uoh.cl), Gabriel Gálvez[1,2], Gladis Serrano[1,2], Samuel Parra[3], Claudia Stange[3], Mauricio Latorre[1,2,4]. [1]Laboratorio de Bioingeniería; Instituto de ciencias de la ingeniería; Universidad de O'Higgins, Rancagua, Chile. [2]Centro de biología de sistemas para el estudio de comunidades extremófilas de relaves mineros (SYSTEMIX), Universidad de O'Higgins, Rancagua, Chile. [3]Centro Biología Molecular Vegetal, Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile. [4] Laboratorio de bioinformática y expresión génica, INTA, Universidad de Chile, Santiago, Chile.

Introduction: Copper mine tailings, with high metal levels and acidic pH, create harsh conditions for most organisms. The Cauquenes tailing, from the El Teniente mine, is one of the country's largest. Our previous studies identified bacterial species in these tailings with potential plant growth-promoting (PGP) traits. This research focuses on isolating copper-resistant bacteria with PGP capabilities to enhance tomato growth.
Materials and Methods: We collected non-redundant native isolates from the Cauquenes tailings and tested them for potential PGP traits, including indole-3-acetic acid (IAA) production, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity, phosphate solubilization, and nitrogen fixation. Their ability to promote tomato growth was evaluated in an *in-vitro* inoculation assay.
Results: All 12 isolates resistant to 10 mM Cu produced IAA and solubilized phosphates. 11 showed ACC deaminase activity, and 10 demonstrated nitrogen fixation. Of those, two isolates enhanced root growth in tomato plants grown *in-vitro*.
Discussion: Our study indicates that the Cauquenes tailings contain bacteria with potential PGP capabilities. These copper-resistant bacteria may be useful for promoting plant growth in copper-contaminated soils, a concern in many agricultural areas.
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114. Role of mitochondrial E3 ubiquitin ligase MUL1 in heart failure with preserved ejection fraction (HEpEF). Angélica Ortega-Muñoz[1] (m.ortegaangelica@gmail.com), Francisco Pino De la Fuente[1,2], Ximena Calle-Chalco[1,3], Mayarling F Troncoso[1,2], Brenda Becerra[1], Claudia Muñoz[1], Alejandra Hernández[1], Francisca Valenzuela[1], Rut Yero-Haber[1], Elsa Rocío Bascuñan[1], Magda C. Díaz-Vesga[1], Douglas Matthies[4], David Silva[1], Alejandra Guerrero-Moncayo[1], Gerald Zapata-Torres[4], Sergio Lavandero[1,5]. [1]Advanced Center for Chronic Diseases (ACCDiS), Facultad de Ciencias Químicas y Farmacéuticas & Facultad Medicina, Universidad de Chile, Santiago, Chile. [2]School of Medical Technology, Faculty of Medicine, University of Chile, Santiago, Chile [3]Institute of Health Sciences, University of O'Higgins, Rancagua, Chile. [4]Molecular Graphic Unit, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile. [5]Cardiology Division, University of Texas Southwestern Medical Center, Dallas, Texas, USA.

Background: MUL1 is an E3 ubiquitin ligase located in the mitochondrial outer membrane with a Ring finger domain that regulates mitochondrial dynamics and function. One of its target proteins is MFN2, which reduces protein levels and, consequently, decreases mitochondrial fusion. MUL1 also SUMOylates DRP1, which stimulates mitochondrial fission, promoting mitochondrial fragmentation involved in cardiovascular diseases. MUL1 promotes cardiac hypertrophy; however, its participation in the development of HFpEF remains unknown.
Objective: To evaluate the effect of cardiomyocyte-specific MUL1 overexpression on myocardial function in HFpEF mice and its in-vitro catalytic inhibition to prevent hypertrophy in neonatal rat cardiomyocytes.
Methods: C57BL/6N wild type (WT) and C57BL/6N-Tg(aMHC-Mul1) mice were assigned to both groups: control diet and high-fat diet (HFD) + L-NAME (1.5 g/L) + NaCl (2%) (HFpEF) for 20 weeks. The bioethical protocol was CICUA-CQyF2022-47. Cardiovascular, functional, and molecular evaluations were performed.
Results: WT HFpEF and C57BL/6N-Tg(aMHC-Mul1) mice showed a significant increase in cardiac MUL1. Diastolic function (E/e), hypertrophy (heart weight/tibia length), blood pressure, and exercise tolerance tests were assessed, revealing significant differences between groups. Using in silico analysis, a peptide was synthesized that could inhibit the catalytic action of MUL1, thereby inhibiting mitochondrial fission in vitro. Two-way ANOVA. A value of $p>0.05$ was considered significant. Mean ± SD, n=7-9 animals per group.
Conclusion: Cardiomyocyte-specific overexpression of MUL1 increases diastolic dysfunction in mice, possibly through changes in mitochondrial dynamics proteins. These findings significantly contribute to our understanding of the role of MUL1 in HFpEF. Future in vitro and in vivo experiments should help elucidate the peptide's effect on cardiomyocyte hypertrophy and myocardial function.

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115. Enzymatic characterization of lactase activity from the probiotic strain *Limosilactobacillus fermentum* UCO-33. Claudia Ortega[1] (clortega2019@udec.cl), María-Belén Reyes[1], Apolinaria García[2], Elena-Amparo Uribe[1]. [1]Laboratory of Enzymology. [2]Laboratory of Bacterial Pathogenicity. Faculty of Biological Sciences, University of Concepción.

Introduction: Lactase is an enzyme that hydrolyzes lactose into galactose and glucose, facilitating its absorption. Various studies propose that some bacterial strains that present this enzyme are probiotic candidates to help in the treatment of lactose intolerance, in addition to providing beneficial effects for the host and modulating the microbiota. Favorable results of lactase activity were found in the strain *L. fermentum* UCO-33, obtained from the microbiota of a patient. In this work, the lactase activities present in this strain were purified and characterized.
Materials and Methods: Genomic analysis of the strain *L. fermentum* UCO-33 was performed. For enzymatic studies, the strain *L. fermentum* UCO-33 was cultured, and a total protein extract was obtained which was chromatographed on anion exchange columns (DEAE-cellulose, Hi Trap Q HP, AKTA start, Cytiva). The fractions were separated, and the kinetic parameters of the activities found were obtained. The enzymatic activity was determined using orthonitrophenyl-betagalactoside (ONPG) as a substrate, Phosphate buffer pH 7.0, at 37°C.
Results: Genomic analysis allowed the identification of two possible coding sequences for lactase, one of 72 KDa and one of 35 KDa. In the chromatographic purifications, 2 peaks with enzymatic activity were detected. The enzyme retained in the chromatography presented a *Km* of 0.1±0.02 mM for ONPG and a specific activity of 2000 U/mg of protein. The 2nd lactase activity detected is still being characterized.
Discussion: Lactase activity was detected in the strain *L. fermentum* UCO-33, the enzyme presents a *Km* comparable with lactases from other probiotics. The amino acid identification of the expressed enzymes and the characterization of the 2nd lactase activity detected are planned.
Acknowledgment: FONDECYT 1230549.

116. The NLP7 transcription factor regulates transcriptomic responses and stomatal-related genes in guard cells to enhance drought resistance in Arabidopsis. Camilo Osorio[1,2] (cosorio@ibio.cl), Nathan Johnson[2,3], Ariel Herrera[1,2], Francisca Blanco[1,2], José Miguel Álvarez[1,2]. [1]Plant Genome Regulation Lab, Centro de Biotecnología Vegetal, Universidad Andrés Bello, Chile. [2]Fundación Instituto de Biología Integrativa, Chile. [3]Centro de Genómica y Bioinformática, Universidad Mayor, Chile.

Introduction: Drought and nutrient availability are crucial for plant growth and productivity. Understanding the interaction between these signals is essential for enhancing drought resilience. The transcription factor NLP7, a master regulator, links nitrogen (N) metabolism genes to drought responses through gene expression changes. Interestingly, the *nlp7* mutant displays a drought resistance phenotype, highlighting the role of nutrient signaling in stress adaptation. However, how NLP7 signaling modulates drought responses is unclear. This research explores the specific function of NLP7 in drought physiological and gene expression responses in guard cells, which form the stomata pore that allows water and gas exchange relevant for drought resistance.
Materials and Methods: Fourteen-day-old *Arabidopsis thaliana* seedlings were transferred to vermiculite or peat-perlite-vermiculite pots and acclimated for 1 or 7 days under long-day conditions. After 14 days of drought stress and rehydration, survival rates were assessed. Stomatal aperture and density were determined from leaf impressions using optical microscopy. Relative water content (RWC) was calculated from rosette biomass parameters at different drought stages. Protoplasts were generated from GFP-labeled stomata plants for cell sorting and RNA-seq. DEGs were identified using DESeq2, and GOstats determined overrepresented biological processes regulated by NLP7 in stomatal tissue.
Results: The *nlp7* mutant showed enhanced drought resistance than Col-0, with higher survival rates and less water loss under high N conditions. Additionally, *nlp7* exhibited less reduction in rosette area and more efficient stomatal regulation, minimizing water loss during stress. Transcriptomic analysis revealed key biological processes regulated by NLP7 in stomatal cells, highlighting *nlp7*'s unique responses under combined drought and N conditions.
Discussion: The findings indicate that NLP7 controls adaptive strategies under drought and N stress, enhancing resilience through efficient stomatal regulation and water conservation. These insights could guide the development of more resilient crops in challenging environments.
Acknowledgment: Instituto Milenio de Biología Integrativa iBio Chile ICN17_002, National Science Foundation NSF IOS-1840761, ANID Fondecyt Regular 1210389.

117. Mic19 expression and sexual dimorphism in an anthracycline-induced cardiac senescence model. Ingrid Oyarzún[1,2], Wileidy Gómez [1,2], Georthan Mancilla[1,2,3,4], Pablo Castro[1,2], Clara Quiroga [1,2], Hugo Verdejo[1,2]. [1]Laboratorio de Señalización Cardiovascular, División de Enfermedades Cardiovasculares, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile. [2]Advanced Center for Chronic Diseases (ACCDiS), Pontificia Universidad Católica de Chile & Universidad de Chile, Santiago, Chile. [3]Laboratorio de Mecanotransducción de señales cardiovasculares. Departamento de Fisiología, Facultad de Medicina, Universidad de Chile. [4]Instituto de Ciencias Biomédicas (ICBM), Facultad de Medicina, Universidad de Chile.

Introduction: The Mitochondrial-Contact-Site-and-Cristae-Organizing-System (MICOS) complex is crucial to maintaining mitochondrial structure and function by shaping and stabilizing cristae junctions and facilitating contact between the inner and outer mitochondrial membranes. Disruptions in MICOS function are linked to mitochondrial dysfunction. MIC19, a subunit of the MICOS, acts as an interconnector between MICOS subcomplexes, underscoring its importance for overall complex functionality. Cardiovascular diseases (CVD) are known to exhibit sexual dimorphism, with aging being a significant risk factor. Our in-silico analysis revealed differentially expressed mitochondrial genes in heart tissue from young and aged male and female rats, with alterations observed in Mic19 and other MICOS components in aged rats. To further explore these findings, we aim to validate the expression of these genes in isolated sexed cardiac cells subjected to a doxorubicin (Dox)-induced senescence protocol.
Materials and Methods: Cardiomyocytes from female and male neonatal rats were treated with 100 nM Dox. The senescence and expression of Mic19 were validated by RT-qPCR and microscopy.
Results: Our analysis demonstrated that Dox induces senescence in cardiomyocytes regardless of sex. However, a significant reduction in Mic19 expression was observed specifically in senescent male cardiomyocytes (30%-72h), while female-derived cells did not exhibit this decline. Notably, these differences in Mic19 expression were evident even at early time points (31%-3h and 14%-24h).
Discussion: This study is the first to reveal a sex-specific differential response in the expression of the MICOS complex, particularly Mic19, during senescence in cardiomyocytes. This finding suggests that beyond the well-established hormonal influences, intrinsic differences in MICOS complex regulation may contribute to the pronounced sex-dependent variations observed in male and female hearts. Our results provide a foundation for proposing novel sex-specific therapeutic or diagnostic strategies for CVD, potentially leading to more personalized and effective treatment approaches for men and women.
Acknowledgment: FONDECYT-1211270 (HV), PUENTE-UC 2024-7 (CQ) and FONDAP 1523A0008 (CQ-PC-HV).

118. Design, recombinant production, and optimization of ASNASE_H_Q, a humanized chimeric asparaginase with biopharmaceutical potential. Pedroso, A.[1,2, 4] (a.pedroso01@ufromail.cl), Lefin, N.[2], Miranda, J.[2], Monteiro, G.[3], Pessoa Jr, A.[3], Farias, J.G [2]., Pedroso, E.[5]. [1]Doctorate Program in Sciences, mention in Applied Cellular and Molecular Biology, Universidad de La Frontera, Chile. [2]Chemical Engineering Department, Science and Engineering Faculty, Avenida Francisco Salazar 01145, Temuco, Chile. [3]Department of Biochemical and Pharmaceutical Technology, School of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil. [4]Departamento de Ciencias Básicas, Facultad de Ciencias, Universidad Santo Tomas, Chile. [5]Hospital de Lota, Departamento de Medicina, Lota ,Bio Bio.

Introduction: Among the drugs used in the chemotherapeutic treatment of acute lymphoblastic leukemia (ALL) is L-asparaginase (ASNase). This enzyme possesses antitumor properties due to its hydrolytic capability and has significantly contributed to the successful treatment of ALL. However, this enzyme carries a strong immunogenic potential, leading to a wide range of adverse reactions, as the commercial variant is of bacterial origin. Hence, discovering new variants with activity and lower immunogenicity stands as a top priority in science. One approach to address this issue is through the design of chimeric enzymes, which could offer a promising solution. This study focuses on obtaining a recombinant humanized variant of asparaginase and optimizing its expression in the periplasm of *Escherichia coli (E. coli).*
Materials and Methods: Eight fragments of *E. coli* ASNase were replaced via bioinformatics to create a chimera. After evaluating its stability and dynamics, a plasmid was designed for expression.
The synthetic gene was amplified, inserted into the pET-22b(+) vector, and introduced into E. coli cells. Resistant cells were selected, and protein expression was induced with IPTG in E. coli strains.
The cells were lysed, and the periplasmic enzyme was extracted and analyzed using SDS-PAGE and enzyme activity assays. A response surface graph was created to optimize expression conditions using Design Expert software.
Results and Discussion: The present study focused on the recombinant expression of a chimeric enzyme generated by substituting the antigenic determinants present in E. coli 3ECA ASNase with regions found in a human enzyme. The E. coli strains used were E. coli BL21 (DE3) and Rosetta (DE3). The results obtained demonstrated the feasibility of designing a humanized chimeric enzyme with fragments from the human variant of the enzyme, using 3ECA as a model. This enzyme could be expressed and exhibited asparaginase activity. However, it's worth noting that the enzyme has not been optimized yet.
Acknowledgment: Doctoral Scholarship ANID 21231090, FAPESP- UFRO 2020/06982-3.

119. Oxidative Stress in Right Ventricular Hypertrophy: High altitude Challenge. Pena Eduardo (Eduardopena@unap.cl). High Altitude Medicine Research Center (CEIMA), Arturo Prat University, Iquique, Chile.

Introduction: High altitude (hypobaric hypoxia) triggers several mechanisms to compensate for the decrease in oxygen bioavailability. One of them is pulmonary artery vasoconstriction and its subsequent pulmonary arterial remodeling. These changes can lead to pulmonary hypertension and the development of right ventricular hypertrophy (RVH), right heart failure and ultimately death. Therefore, the aim is to describe the molecular pathways -including oxidative stress, inflammation, protein kinases activation and fibrosis- and

determine the proteins associated in the development of cardiac hypertrophy under this condition, finally propose the current therapeutic approaches for these conditions.
Results: The signaling pathways involved in the RVH under hypoxia conditions are related to oxidative stress (Nox-derived O_2.- and H_2O_2), protein kinase (ERK5, p38α and PKCα) activation, inflammatory molecules (IL-1β, IL-6, TNF-α and NF-kB) and transcription factor (HIF-1α). On the other hand, rats with long-term CIH-induced RVH clearly showed Nox2, p22phox and LOX-1 upregulation and increased lipid peroxidation, HIF-1α stabilization and p38α activation.
Discussion and Conclusion: Levels of lipid peroxidation measures have been described as indirect but effective indexes of ROS generation. Moreover, lipid peroxidation interacts with kinases, trigger inflammatory molecules involved in cardiac hypertrophy. In conclusion, Nox2, p22phox and LOX-1 upregulation and increased lipid peroxidation, HIF-1α stabilization and p38α activation may be considered new targets in CIH-induced RVH. Finally, recent therapeutic approaches have focused on abolishing hypoxia-induced RVH and RHF via attenuation of oxidative stress and inflammatory pathways through administration of natural products, such as astaxanthin.
Acknowledgment: FONDECYT-iniciación (11230214).

120. Secreted Salivary Phospholipase D of *Myzus persicae* Interferes in Defense Pathways And Promote Plant Susceptibility Against Pathogen Attack. Micaela Peppino Margutti[1,2,3] (peppinomarguttim@gmail.com), Brunella Carpio Balarezo[1,], Maria Francisca Blanco[1,2,3]. [1]Universidad Andrés Bello, Centro de Biotecnología Vegetal, Facultad de Ciencias de la Vida, Santiago 8370186, Chile. [2] Millennium Science Initiative Program (ANID), Millennium Institute for Integrative Biology (iBio), Santiago, Chile. [3]Millennium Science Initiative Program (ANID), Millennium Nucleus for the Development of Super Adaptable Plants (MN-SAP), Santiago, Chile.

Aphids, as one of the most destructive pests globally, inflict significant crop losses. In their interaction with the host, aphids secrete effectors through their stylet to inhibit Herbivore-associated molecular patterns-triggered immunity (HTI) and reduce immune response via effector-triggered susceptibility (ETS). Among the enzymes involved in HTI activation are phospholipases. Plant phospholipases play a crucial role in membrane remodeling and modulate second messengers, which are vital in defense responses during biotic stress. While multiple pathogens synthesize phospholipases to compete with host enzymes, the function of aphid phospholipases as effectors during infestation has never been demonstrated. Through bioinformatics approaches, we identified a Phospholipase D of *Myzus persicae* in aphid saliva (Mp-PLD) that shares certain features with effectors. To confirm the predicted subcellular localization, we did a construction of Mp-PLD:GFP controlled by 35S promoter. Our findings revealed that the protein is localized to the membrane and Nicotiana cell nuclei. We also demonstrated that overexpression of Mp-PLD alters the expression of defense-related genes and genes implicated in membrane

remodeling. Furthermore, we measured H_2O_2 production and chlorophyll levels and observed changes in both parameters in plants overexpressing Mp-PLD compared with the control. These results suggest that Mp-PLD could act in plant cells and interfere with defense pathways, potentially promoting plant susceptibility.
Acknowledgment: ANID-FONDECYT Regular 1210320 and Posdoctorado 3240308, Programa Iniciativa Científica Milenio-ICN17_022, NCN2021_010, and ANID PIA/BASAL FB0002.

121. Biochemical characterization of the trifunctional ThiDN enzyme from *Pyrococcus furiosus*, a key enzyme in vitamin B1 biosynthesis. Martín Pereira-Silva (martin.pereira@ug.uchile.cl), Nicolás Fuentes-Ugarte, Victoria Guixé and Víctor Castro-Fernández. Laboratorio de Bioquímica y Biología Molecular, Faculta de Ciencias, Universidad de Chile, Santiago, Chile.

In archaea and bacteria, the biosynthesis of thiamine phosphate (THI-P) involves the formation of 4-amino-5-hydroxymethyl-2-methylpyrimidine pyrophosphate (HMP-PP) and 4-methyl-5-β-hydroxyethylthiazole phosphate (THZ-P), which are condensed by enzymes with thiamine phosphate synthase activity, generating THI-P. The synthesis of HMP-PP involves two consecutive phosphorylation of 4-amino-5-hydroxymethyl-2-methylpyrimidine (HMP). First, HMP is phosphorylated to HMP-P (HMPK activity) and subsequently to HMP-PP (HMPPK activity). Both phosphorylation reactions occur at the same active site of enzymes encoded by the *thiD* gene. On the other hand, in archaea, THI-P is formed by the action of thiamine phosphate synthase activity encoded by the *thiN* gene, which is not homologous to the bacterial gene. Interestingly, in some extremophilic archaeal organisms like *Pyrococcus furiosus*, the *thiD* and thiN genes are fused, forming the thiDN gene, and encoding the ThiDN protein, which should have three enzyme activities: HMPK, HMPPK, and thiamine phosphate synthase.
In this work, we characterized the kinetics and thermostability of the putative trifunctional enzyme of *Pyrococcus furiosus* (PfThiDN). We developed an enzymatic coupled assay using auxiliary enzymes from thermophilic organisms. We used the ADP-dependent glucokinase enzyme from *Thermococcus litoralis* (TlGK) and the $NADP^+$-dependent glucose-6-phosphate dehydrogenase from *Thermotoga maritima* (TmG6PDH) to measure the HMPK/HMPPK kinase activity by following the reduction of $NADP^+$ to NADPH at 340 nm. We used this assay to determine the PfThiDN kinetics parameters (K_M and V_{max}) to 70 °C. In addition, we identified a principal oligomeric state corresponding to a trimer by size exclusion chromatography.
Through X-ray crystallography, we obtained a crystal structure of PfThiDN at 3.26 Å that describes the overall organization of the ThiD and ThiN domains, besides the presence of the HMP-P and $ADP_{\beta S}$ at the active site of the ThiD domain, being the first structure reported for this trifunctional enzyme.
Funding: Fondecyt 1221667.

122. Makomakine: Bifunctional indole alkaloid that inhibits NF-κB and protects against oxidative stress to treat alzheimer's disease and cellular senescence. Perez, R (perezcolladorebeca@gmail.com), Camins, A., Paz, C. Laboratory of Natural Products & Drug Discovery, Department of Basic Science, Center CEBIM, Universidad de La Frontera, Temuco, Chile.

Introduction: Alzheimer's disease is the most prevalent neurodegenerative dementia, linked to chronic inflammation, oxidative stress and cellular senescence. Accumulation of beta-amyloid and neurofibrillary tangles, together with reactive oxygen species, activates the NF-κB pathway, promoting neuroinflammation and neuronal degeneration. Cellular senescence, a state in which cellsacquire a proinflammatory secretory phenotype (SASP) contributes to a proinflammatory environment, exacerbating the disease. Identifying compounds that inhibit NF-κB and protect againstoxidative stress may reduce the release of inflammatory factors associated with SASP, this being crucial to develop effective therapies against Alzheimer's disease. *Aristotelia chilensis*, a native Chilean tree, produces indole alkaloids with potential neuroprotective properties. This study evaluates the neuroprotective activity of Makomakina, an alkaloid isolated from *A.chilensis* against proinflammatory and prooxidant stimuli in HMC-3 microglial cell line and primary C57BL/6 mousecultures.
Materials and Methods: Cultures were pretreated with 50 µM Makomakin for 3 hours, followed byincubation with 100 ng of LPS for 2 hours, phosphorylation of IκB was assessed by western blot. In addition, in primary cultures of C57BL/6 embryo cortex, anti-inflammatory activity was determinedby immunocytochemistry with Rabbit-anti-GFAP and Mouse-anti-B-tubulin primary antibodies andto assess antioxidant activity alkaloids were added at concentrations from 6.25 to 200 µM for 1 hour,followed by treatment with 2 mM H_2O_2 for 24 hours, protective activity was measured by MTT assay.
Results: Makomakine protects from H_2O_2 -induced death of mixed primary culture brain cells by 100% at a concentration of 6.25 µM, in microglia cells it causes a significant reduction in the levels of phosphorylated IκB (p-IκB) compared to the control group, as demonstrated by Western blot.
Acknowledgment: Projects: FONDECYT 1220831, ANID - FONDEQUIP EQM220161, ANID National Doctorate Scholarship N° 21220534.

123. Two types of physical training improve diastolic function in an experimental HFpEF model. Francisco Pino-De la Fuente[1,2] (pinodelafuente.francisco@gmail.com) Angelica Ortega-Muñoz[1], Mayarling F Troncoso[1,3], Claudia Muñoz[1], David Silva[1], Danica Jiménez-Gallegos[1], Raúl Flores[1], Sebastián Urquiza-Zurich[1] Ximena Calle-Chalco[1,4], Francisca Valenzuela[1], Elsa Rocío Bascuñán[1], Alejandra Hernández[1], Alejandra Guerrero-Moncayo[1], Magda C. Díaz-Vesga[1] Rodrigo Troncoso[1,2], Sergio Lavandero[1,5]. [1]Advanced Center for Chronic Diseases (ACCDiS), Facultad de Ciencias Químicas y Farmacéuticas & Facultad Medicina, University of Chile, Santiago, Chile. [2]Institute of Nutrition and Food

Technology (INTA), University of Chile, Santiago, Chile. [3]School of Medical Technology, Faculty of Medicine, University of Chile, Santiago, Chile. [4]Institute of Health Sciences, University of O'Higgins, Rancagua, Chile [5]Cardiology Division, University of Texas Southwestern Medical Center, Dallas, Texas, USA.

Introduction: Patients with heart failure with preserved ejection fraction (HFpEF) exhibit diastolic dysfunction due to increased stiffness and decreased relaxation capacity of the ventricular wall. HFpEF patients also experience glucose metabolism impairment, reduced exercise tolerance, and diminished quality of life. Our collaborative work with Dr. Hill´s group allowed us to develop the first experimental HFpEF model.
Objective: To determine whether high-intensity interval training (HIIT) or resistance training (RT) improves diastolic dysfunction in HFpEF mice.
Methods: C57BL/6N mice were divided into the following groups: a) control diet (CD), b) high-fat diet (HFD) plus L-NAME (1.5 g/L) (HFpEF), c) HIIT+HFpEF and d) RT+HFpEF for 20 weeks (Bioethics protocol: 22537–CQyF–UCH). Evaluations were conducted at the end of the treatment period. Data are shown as mean ± SD, n=10-11 animals per group. Statistical analysis: ANOVA one-way. A value of $p<0.05$ was considered significant.
Results: Mice treated with HFD plus L-NAME completely reproduced the HFpEF phenotype, including hypertension, diabetes, preserved systolic function, cardiac hypertrophy and diastolic dysfunction (increase in E/e´), and exercise intolerance. Cardiac variables showed that animals trained with both types of exercise exhibited a lower E/e' ratio and improved glucose metabolism. Mice trained with RT showed enhanced muscular endurance and running capacity.
Conclusion: Both types of training in HFpEF mice prevent the progression of cardiac pathology, improving glucose metabolism and physical performance. Therefore, physical training with both types of exercise may be an effective therapy for treating this pathology.
Acknowledgment*:* FONDECYT 1240443 (SL), FONDAP 15130011 & 1523A0008 (SL) and ANID PhD fellowship 21200953 (FPDLF).

124. FchDOF1 and FchNAC2 participate in a coordinated way in ripening regulation of *Fragaria chiloensis* fruit. Nicole Pizarro-Vásquez (npizarro@alumnos.utalca.cl), Macarena Zamorano-Curaqueo, Raúl Herrera, María Alejandra Moya-León. Functional Genomics, Biochemistry and Plant Physiology Group, Instituto de Ciencias Biológicas, Universidad de Talca.
Introduction: Transcription factors (TFs) play important roles during fruit ripening, regulating and coordinating the expression of genes involved in cell wall disassembly. In *Fragaria chiloensis* fruit the transcription of several TFs increases as the fruit ripens, such as *FchDOF1* and *FchNAC2* among others. The aim of this work is to analyze the participation of these two TFs in the transcriptional regulation of genes related to softening of *F. chiloensis* fruits such as *FchPL* and *FchEXP2*.
Materials and Methods: Developing *F. chiloensis* fruits were agro-infiltrated with an overexpression vector containing the *FchDOF1* sequence, the *FchNAC2*, both sequences

or the empty vector (EV). Fruit tissues were collected after three days, frozen and stored at -80°C. Total RNA was extracted, cDNA synthesized, and the level of transcripts quantified by RT-qPCR. Bimolecular fluorescence complementation (BiFC) and Yeast-two hybrid (Y2H) assays were employed to evaluate the interaction between the TFs. Luciferase-dual assay was performed for evaluate transactivation activity of TFs on selected gene promoters.
Results: *FchDOF1* transcripts increase along *F. chiloensis* development and ripening. *FchDOF1* overexpressing fruits display a rise in red color (color a) compared to EV fruit. Gene expression analysis revealed an increment in transcript levels of *FchNAC2* and *FchPL* in *FchDOF1* overexpressing fruits. BiFC and Y2H assays confirm interaction between FchDOF1 and FchNAC2 proteins. Luciferase dual assays indicate that FchDOF1 transactivates *FchPL´*s promoter but not that of *FchEXP2*. Interestingly the presence of both FchDOF1 and FchNAC2 is required to transactivate *FchEXP2´*s promoter.
Discussion: These results suggest that FchDOF1 and FchNAC2 interact in a coordinated manner in the transcriptional regulation of genes related to the ripening of *F. chiloensis fruits*.
Acknowledgment: FONDECYT 1210948 and ANILLO ACT210025 grants.

125. Expression and Purification of Mouse TRPM4 and TRPM8 in *Saccharomyces cerevisiae*. Poni, K., Yevenes, A., González-Nilo, F. Center for Bioinformatics and Integrative Biology, Universidad Andrés Bello.

Transient Receptor Potential Melastatin (TRPM) channels, particularly TRPM4 and TRPM8, are vital in multiple physiological processes. Human TRP channels are widely expressed throughout the body, including brain, heart, skin, pancreas, as well as in inflammatory and immune cells. TRPM4 is a Ca^{2+}-activated monovalent cation channel that affects neuron and cardiomyocyte excitability and chemosensory activity in taste receptor cells. It regulates membrane potential, crucial for cellular function, so the dysregulation of TRPM4 is associated with conditions such as cardiac arrhythmias and neurodegenerative diseases. Conversely, TRPM8 is a non-selective cation channel activated by cold temperatures and cooling agents, like menthol. It plays a key role in thermosensation and pain perception, underscoring its importance in hemostasis, making it a target for developing analgesics and treatments for chronic pain. Being membrane proteins, many types of studies of TRP channels are nevertheless hindered by the difficulty of producing protein samples of sufficient quantity and quality in an economically sustainable manner. To study these channels, we developed a method for their expression and purification in *Saccharomyces cerevisiae* as fusion proteins with Green Fluorescent Protein (GFP). Using yeast as a host organism offers advantages like ease of genetic manipulation, its relevance as a eukaryotic model system, and cost-effective large-scale protein production and the GFP fusion allows for easy monitoring of expression levels. Our method involves cloning the mouse TRPM4 and TRPM8 genes into a yeast expression vector pYES2 (Thermo), incorporating the Tobacco Etch Virus (TEV) protease cleavage site (ENLYFQ\S), GFP, and eight histidine residues at their C-terminal through homologous recombination. Purification is done via

affinity chromatography. Then, the purified proteins undergo calorimetric analysis to assess their thermal stability and interaction with potential ligands. Preliminary results show successful expression and purification of both TRPM4-GFP and TRPM8-GFP fusion proteins, with distinct calorimetric profiles for each channel. This study demonstrates the feasibility of using *S. cerevisiae* for producing functional TRPM channels and lays the groundwork for further biophysical and pharmacological research. This approach opens new avenues for understanding these channels' roles in health and disease, potentially leading to novel therapeutic strategies.
Acknowledgment: FONDECYT 1221498.

126. Study of the physiological and molecular response to drought on two different genotypes of the genus *Eucalyptus*. A. Carolina Puentes-Romero[1](anacaropuentes@gmail.com), Constanza Frois-Mesa[1], Paulo Cañete-Salinas[2], Felipe Valenzuela-Riffo[1], Ma. de los Ángeles Contreras[1], Raúl Herrera[1]. [1]Instituto de Ciencias Biológicas, Universidad de Talca. [2]Facultad de Ciencias Agrarias, Universidad de Talca.

Introduction: Currently, forests are being negatively affected by climate change, due to greater water stress owing to the reduction in water resources. The decrease in precipitation requires the selection of drought tolerant genotypes. In Chile, *Eucalyptus* spp. are the second important in planting areas for the forest industry, however, these species have been severely affected by drought, limiting their yield and affecting wood quality. Furthermore, the knowledge of their physiological and molecular responses to drought remains limited. The aim of this work was to compare the physiological and molecular response to drought of two genotypes from *Eucalyptus* (G1 and G2).
Materials and Methods: G1 and G2 seedlings were stressed by the absence of irrigation for 14 days. Measurements of physiological parameters were performed. Also, several housekeeping genes were evaluated and selected for qRT-PCR studies. Moreover, a genome-wide identification was carried out for genes involved in stomatal closure, as their relative expression.
Results: G2 exhibited a sustained and gradual reduction in its stomatal conductance under water stress conditions with a reduction of 12% on day 7 and of 95% from day 7 to 14, while G1 perceived an increase of 160% on day 7, to subsequently fall sharply by 93% from day 7 to 14, showing greater susceptibility after 7 days of drought. Three housekeeping genes *GAPDH*, *IDH* and *H2B* showed stable expression in leaves during drought. The analysis of the stomatal closure genes: *PYL5*, *QUAC1* and *SLAC1* showed differential expression at 7 and 14 days without irrigation. Furthermore, their gene families were structurally characterized and positioned in the *Eucalyptus* genome.
Discussion: Together, the results showed a better performance of G2 genotype under prolonged drought. Results allow better decisions to be taken when planning a commercial plantation under water deficit conditions.

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127. Endothelin-1 enhances ROS production in gallbladder cancer cells. Nelson Quilaqueo Millaqueo[1] (nelson.quilaqueo@alumnos.uach.cl), Diego Vera[1], Claudia Quezada[1], José Sarmientos[2], Ignacio Niechi[1]. [1]Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile. [2] Instituto de Fisiología, Facultad de Medicina, UACh, Valdivia, Chile.

Introduction: Gallbladder cancer (GBC) is a highly malignant tumor with a poor prognosis, often diagnosed incidentally. The invasive capacity of tumor cells is regulated by molecules such as endothelin-1 (ET1), which is linked to the transformation of cells into an invasive phenotype though ET_A receptor (ET_AR). Reactive oxygen species (ROS), primarily produced by mitochondria and the NADPH oxidase (NOX) protein family, act as second messengers promoting invasiveness. In endothelial cells, ET1 increase ROS levels by activating NOX proteins, however, the relationship between ET1/ET_AR and NOX in tumor cells is still not well understood, which is the focus of this study.
Material and Methods: GBC cell line NOZ was used and treated with ET1 and/or Ambrisentan (ET_AR antagonist). Live cell imaging was carried to measure total and mitochondrial ROS with the fluorescent probes DCFDA and Mitosox Green respectively using spinning disk confocal microscopy. Additionally, NOX1 protein levels were determined by western blot.
Results: Treatment with ET1 led to an increase in total ROS levels, which was blocked by Abrisentan Moreover, mitochondrial ROS was not affected by ET1 signaling modulation. Finally, ET1/ET_AR increases NOX1 protein levels.
Discussion: ET1/ETAR signaling enhances non-mitochondrial ROS levels, and this increase is associated with the upregulation of NOX1 protein in gallbladder cancer cells.
Acknowledgment: ANID/FONDECYT Fondecyt de iniciación 11220149; FONDEQUIP EQM 1501118.

128. Digital processing algorithm for immunohistochemical analysis of breast carcinoma markers. Aracelly Quiroz[1] (aracellyquiroz@udec.cl), Susana Pineda[1,2], Carolina Delgado[1,2], Paola Bazzoni[3], Norma Tolaba[3], Gonzalo Sequeira[3], Ignacia Marín[4], Carla Alvares[4], Robinson López[4]. [1]Sección de Patología, Departamento de Especialidades, Facultad de Medicina, Universidad de Concepción, Chile. [2]Unidad de Patología, Hospital Guillermo Grant Benavente Concepción, Chile. [3]Hospital Dr. Arturo Oñativia, Ciudad de Salta, Argentina. [4]Carrera de Tecnología Médica, Universidad San Sebastián, Concepción, Chile.

Introduction: Immunohistochemistry (IHC) is as a diagnostic instrument for breast cancer, using the analysis of Estrogen Receptor (RE), Progesterone Receptor (RP), HER2, and Ki67. Despite its benefits, a limitation lies in the interobserver variability and the time required

for analysis. In response, we have developed two algorithms for the digital processing of tumor biopsies. These rely on nuclear or membrane segmentation, enabling the extraction of intensity stain and proportional score for RE, RP, Ki67 and HER2. The aim of this study is to evaluated the concordance between our digital pathology algorithm and the pathology routine observation.
Materials and Methods: We selected IHC samples from 100 breast cancer patients to validate our algorithm. These samples were scanned using Easy-Scan (MOTIC) and then assessed with Fiji and QuPath, utilizing 3 regions of interest (ROI) per sample. For mean intensity, we used values of 0, +1, +2, or +3; While for the positive proportion, we used the percentage of positivity. Following this, we examined the agreement between our algorithm and the breast cancer markers index based on observations from 3 pathologists.
Results: Fiji and Qupath used different methods to present results, with Fiji using pixel area and Qupath using a percentage of positive cells. The proportion of Ki67 positive cells varied between Qupath and Fiji due to variability in Ki67 expression in the hot spot zone. Consistency was higher between Qupath and the biopsy report and pathologist's analysis from Chile and Argentina than with Fiji. For better agreement, the ROI in all markers should be initially selected by pathology.
Discussion: We have created a cost-effective, semi-automated method for breast carcinoma evaluation in routine practice using Qupath. This algorithm reduces the time needed for pathological examination, making it a practical approach to analyzing breast cancer markers.

129.cEffect of poly(dA:dT) tracts and Rsc3/30 binding site on the nucleosome remodeling activity of RSC1 and RSC2 complexes. Fernanda Raiqueo[1] (fraiqueo2016@udec.cl), Roberto Amigo[1], David Figueroa[2], Francisco Salinas[2] and José. L. Gutiérrez[1]. [1]Laboratorio de Regulación Transcripcional, Facultad de Ciencias Biológicas, Departamento de Bioquímica y Biología Molecular, Universidad de Concepción, Concepción, Chile. [2]Laboratorio de Genómica Funcional, Facultad de Ciencias, Instituto de Bioquímica y Microbiología, Universidad Austral de Chile, Valdivia, Chile.

Introduction: The nucleosome is the basic unit of chromatin. Nucleosomes usually block the access of proteins to their binding sites, affecting processes such as transcription. Chromatin opening by chromatin remodeling complexes (CRCs) is an ongoing process at gene promoters that allow transcription. Depending on the direction of nucleosome displacement in the DNA chain, binding sites can be exposed or occluded. To provide the correct outcome, CRCs must know where to displace nucleosomes. An essential CRC in *Saccharomyces cerevisiae* is RSC. How RSC is recruited to specific genomic regions is still unclear. Poly(dA:dT) tracts and GC-rich binding motifs are believed to orient RSC binding and directionality, but has yet to be proved. RSC is present in 2 subtypes, termed RSC1 and RSC2. The presence of Rsc3 and Rsc30 (Rsc3/30) subunits is more pronounced in RSC1. Rsc3/30 have GC-rich binding motifs, presumed to guide RSC's binding. Little is known

about RSC1's properties, because most studies have focused on RSC2. Recently we found that poly(dA:dT) sequences stimulate RSC2's activity. Whether these tracts stimulate RSC1 and if Rsc3/30's binding site stimulate both RSC complexes, remains unknown.
Materials and Methods: RSC1 and RSC2 were purified from TAP-tagged *S. cerevisiae* strains. The effect of poly(dA:dT) tracts and Rsc3/30's binding sites on RSC complexes was determined by *in vitro* remodeling assays and by *in vivo* reporter assays.
Results: RSC1 and RSC2 were successfully purified. The sliding activity of both RSC1 and RSC2 is enhanced by the presence of poly(dA:dT) to a similar extent. The Rsc3/30 binding site displayed a small reproducible stimulatory effect, although the results were not conclusive.
Discussion: Our results raise the possibility that poly(dA:dT) tracts and Rsc3/30's binding site orient RSC's binding to nucleosomes. This effect, in turn, would establish the direction in which nucleosomes are displaced from the moment of RSC binding.
Acknowledgment: Universidad de Concepción VRID-Investigación 2023000737INV.

130. Impact of Non-Synonymous SNVs in the ORF of Nucleocapsid protein on the Gene Expression of *Orthohantavirus Andesense* (ANDV). Hade Ramos Acevedo[1,4], Andreas Schüller[1,2], Jorge Vera-Otarola[3], Jenniffer Angulo[1,4]. [1]Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago de Chile. [2]Instituto de Ingeniería Biológica y Médica, Pontificia Universidad Católica de Chile, Santiago de Chile. [3]Unidad de Virología Aplicada, Dirección de Investigación de la Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago de Chile. [4]Laboratorio de Infectología y Virología Molecular, Departamento de Enfermedades Infecciosas e Inmunología Pediátrica, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago de Chile.

Introduction: The Andes virus (ANDV), a rodent-borne Hantavirus, causes hantavirus cardiopulmonary syndrome (HCPS) in Chile and Argentina. The ANDV genome comprises three single-stranded negative-polarity RNA segments, large (L), medium (M), and small (S). The Small mRNA (SmRNA) encodes for the nucleocapsid protein (N) and the NSs protein. The N protein is crucial in several stages of viral replication, including SmRNA translation, dependent on the SmRNA 3'UTR. It is known that the N protein interacts with eIF4G, though the interaction mechanism is unknown. Cellular proteins like Mex3A, suggested but not confirmed to interact with N, also play a role. This study investigated the impact of non-synonymous single nucleotide variations (SNVs) previously reported in the N protein's ORF from complete genome sequencing of ANDV variants collected from infected humans and viral isolates.
Methods: Point mutations previously described in the literature (A21T, A21V, N46S, and L247S), were introduced by site-directed mutagenesis into the plasmid ANDV-His-N and confirmed by Sanger sequencing. Molecular docking using the HDOCK web server was performed to identify potential sites between N and eIF4G. The impact of wild-type or mutant

N proteins on gene expression was evaluated using a capped SmRNA viral-like reporter in HEK293T cells.
Results: The A21T and A21V mutants significantly enhanced SmRNA reporter translation, similar to the wild type. However, mutations N46S and L247S disrupted this function, losing the ability to stimulate translation. Molecular docking revealed interaction sites between N and eIF4G, with key interactions near residue 247.
Discussion: This research identifies regions of the N protein that influence SmRNA translation, underscoring the need for further study of ANDV variants and protein structures to better understand the virus's pathogenicity. Identifying specific interaction sites between N and eIF4G also presents new targets for drug development, offering potential therapeutic interventions against ANDV infection.
Acknowledgment: FONDECYT 1230718.

131. Dissecting the Balance Between Lipoic Acid and Ethylene in Senescent Tomato Leaves Through the Application of 1-MCP. Paulo Retamales Vilches (paulo.retamales@ug.uchile.cl), Felipe Uribe Cárdenas, Michael Handford. Centro de Biología Molecular Vegetal, Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile.

Introduction: Lipoic acid (LA) is an important antioxidant in plants that plays an essential role in mitigating abiotic stress. LA is an essential enzymatic cofactor for a set of enzymes, including Pyruvate dehydrogenase and α-Ketoglutarate dehydrogenase, both of which are important for cellular respiration. Interestingly, LA synthesis interacts with the synthesis of ethylene, a key hormone in regulating leaf senescence in plants. Both compounds share S-adenosylmethionine (SAM) as an intermediate in their metabolic routes, suggesting a connection between the antioxidant response and hormonal regulation of senescence. The objective of this study is to evaluate at the molecular level the effects of altering ethylene and LA synthesis pathways on the senescence and maturation of fruits in *Solanum lycopersicum* (tomato) by applying 1-methylcyclopropene (1-MCP), an inhibitor of ethylene perception.
Materials and Methods: Tomato plants (MicroTom variety) were treated with SmartFresh (1-MCP, 1 ppm, 16 h) and harvested after 4 days. Senescent leaves are being molecularly analyzed to determine the mRNA levels of genes in the LA and ethylene synthesis pathways using real-time PCR, using treated mature leaves as controls.
Results: The results to date show that senescent leaves treated with 1-MCP exhibit a decrease in transcript levels of ethylene synthesis genes (*ACO1* and *ACS2*) compared to untreated ones.
Discussion: Our results suggest that inhibiting ethylene perception reduces the synthesis of this hormone, a feature that will be useful in determining the presence of a potential balance between LA and ethylene in the leaf senescence process.

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132. Analysis of potential Mn^{2+} coordinating ligands in agmatinase-like protein (ALP).
María-Belén Reyes[1] (marireyesc@udec.cl), Allison Fuentes[1], Diego Bustamente[1], Fernando Retamal[1], Marcell Gatica[1], José-Yamil Neira[2], Maximiliano Figueroa[1], José Martínez-Oyanedel[2], Elena-Amparo Uribe[1]. [1]Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas. [2]Departamento de Análisis Instrumental, Facultad de Farmacia. Universidad de Concepción.

Introduction: Agmatine is the decarboxylated product of L-arginine and has hypoglycemic, anticonvulsant and antidepressant effects in humans. We identified a novel mammalian protein that hydrolyzes agmatine to putrescine and urea, agmatinase like protein (ALP). ALP differs in its amino acid sequence from prokaryotic agmatinases and does not contain the typical residues that stabilize the activating metal Mn^{2+}. We generated a structural model of ALP and proposed new ligands for the Mn^{2+} cofactor, from which five single mutants were generated.
Materials and Methods: Single mutants were obtained by site-directed mutagenesis and verified by automatic sequencing, they were expressed in Escherichia coli BL21 (DE3) and purified by chromatographic methods. The kinetic parameters were determined at 37°C, pH 9.5 under initial rate conditions.
Results: Single mutants were generated: N213A, Q215A, D217A, E288A, K290A. To analyze the enzyme-Mn^{2+} interaction, activation, dialysis, hyperactivation, determination of metal content and metal activation specificity were performed and in all the results the single mutants did not show differences with the wild enzyme. However, the *Km* decreased in four variants to a third of its value (N213A, Q215A, D217A, E288A), and the *Vmax* normalized by protein concentration decreased to 15, 30, 35 and 20% respectively. In the mutant K290A no decrease in *Km* was observed.
Discussion: The results indicate that four of the mutated residues are located in the vicinity of the active site of ALP, given the alteration in its kinetic parameters, but they would not be especially relevant in the interaction with the activating metal.
Acknowledgements: FONDECYT 1230549.

133. Development of a molecular approach for the detection of *Helicobacter pylori* in formalin-fixed and paraffin-embedded gastric resections.
Joaquín Reyes-González (joaquin.reyes.g@ug.uchile.cl), Antonia Geisse, Troy Ejsmentewicz, Constanza Cárcamo, Samuel Jorquera, Francisca Vidal, Franz Villaroel-Espindola. Translational Medicine Unit, Fundación Arturo López Pérez. Santiago, Chile.

Introduction: *Helicobacter pylori (HP)* is a risk factor for gastric cancer (GC). The detection of the bacteria within the community consists of tests based on fresh samples (*HP*-related antigens or genes), including breath test (urease activity). Currently, *HP* has been involved

in the therapeutic options and clinical outcome of GC patients. This work aimed the implementation of a PCR tool to detect *HP* in formalin-fixed paraffin-embedded (FFPE) tissue from surgical resections of gastric cancer patients.
Materials and Methods: Primers were designed for the UreC gene of *HP.* PCRs with several annealing temperatures were performed to determine the optimal thermal cycling. The sensitivity and specificity were verified using 66 FFPE samples collected by upper endoscopy of non-oncological cases, assessed by OLGA score and confirmed by GIEMSA staining and ELISA test.
Results: The sensitivity (Se) and specificity (Sp) measured using GIEMSA (N=62) as reference was 45% and 41%, respectively; when ELISA (N=66) was used as reference, the results were 51% and 65%, respectively. When true cases were double positive for Giemsa and ELISA (19 out of 41), the PCR Se and Sp were 47% and 68% and it represented a frequency of positivity of 58%.
Discussion: Based on the observed number of false negative cases, our PCR has some limitations related to its positive and negative predictive values (PPV and NPV). The procedure needs to improve both sensitivity and limit of detection. Another reference material and gold standard may be required.
Acknowledgment: ANID-FONDECYT 122141; FALP-LMT-2023&2024.

134. The enzymatic activity of human glucose-6-phosphate dehydrogenase (*h*G6PDH) is modulated by oxidative modifications triggered by peroxyl radicals. Reyes J.S. (juan.reyesv06@gmail.com), Fuentes-Lemus E., López-Alarcón C. Facultad de Química y de Farmacia, Pontificia Universidad Católica de Chile.

Introduction: G6PDH activity is important for cellular redox homeostasis by contributing to the maintenance of the NADPH levels required for glutathione recycling. Despite the well-established interplay between G6PDH activity and oxidative stress-related conditions, it is unknown whether G6PDH is susceptible to oxidative modification and inactivation. Considering the abundance of this enzyme and its structure, we hypothesized that *h*G6PDH is susceptible to oxidative modification and inactivation by the biologically relevant peroxyl radical species (ROO□).
Materials & Methods: *h*G6PDH was incubated with 10 mM AAPH (2,2′-azobis(2-methylpropionamidine)dihydrochloride), at 37 ºC for 3 hours. Enzymatic activity was determined following NADPH. Structural modifications were examined by SDS-PAGE and circular dichroism. Thiols were determined by the DTNB assay, and exposure of hydrophobic sites was determined using the ANS dye.
Results & Discussion: Oxidation of *h*G6PDH with 10 mM AAPH resulted in a significant loss of enzymatic activity (86.63 □ 2.46%). This was accompanied by a decrease only in the catalytic constant of the enzyme by 53%. SDS-PAGE analyses showed loss of the monomeric band with formation of high-molecular mass aggregates. Oxidation of Cys residues seemed to play a significant role in G6PDH cross-linking as these were consumed

by 55.23 □ 6.52% when G6PDH was incubated with 10 mM AAPH. The ANS fluorescence increased by approximately 90% correlating with the loss of secondary structures and a decrease in soluble protein. These results show that ROO□-mediated *h*G6PDH inactivation is associated with thiol consumption, changes in molecular mass.
Conclusion: *h*G6PDH is susceptible to inactivation mediated by ROO□ thought process that included modification of specific residues, change in molecular mass. These changes could be relevance in the formation of intracellular NAPDH
Acknowledgment: Fondecyt 1220459 (CLA), 3220507 (JSR).

135. Altered Calcium Signaling and Apoptosis in POMC Neurons induced by High-Fat Diet. Jorge Ríos[1], Carola Bruna[2], Estefanía Tarifeño-Saldivia[1]. [1]GearLab, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Chile. [2]Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Chile.

Introduction: POMC neurons in the hypothalamus play a crucial role in regulating satiety. Previous studies have shown that obesity induced by diets high in saturated fats compromises the anorexigenic function of these neurons. This research aims to identify transcriptomic changes in POMC neurons induced by an obesogenic diet.
Material and Methods: Using transgenic C57BL/6 Pomc:eGFP mice, fed with a diet rich in saturated fats, alterations in gene expression through RNA-seq in purified POMC neurons were investigated.
Results: Our findings indicate an increased expression of pro-apoptotic factors in POMC neurons from obese mice, suggesting the activation of mitochondrial-mediated intrinsic neuronal apoptosis. Additionally, the overexpression of genes that inhibit apoptosis, along with an upregulation of genes associated with calcium release from the endoplasmic reticulum was observed. Concurrently, a decrease in the expression of genes involved in exocytosis was observed.
Discussion: These results suggest that an obesogenic diet may trigger altered calcium signaling and mitochondrial dysfunction, potentially leading to the activation of apoptosis in POMC neurons. Furthermore, the resulting synaptic dysfunction may impair neuronal communication, exacerbating the dysfunction of POMC neurons and compromising their role in satiety regulation.
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136. Role of Glutamate Receptor-like (GLR) ion channels in the development of the moss Physcomitrella patens during salt stress. Natalia Rivera[1](natalia.rivera@alu.ucm.cl), Monica Yañez[2], Erwan Michard[2]. [1]Escuela de Ingeniería en Biotecnología, Facultad de Ciencias Agrarias y Forestales, Universidad Católica del Maule. [2] Instituto de Ciencias Biológicas, Universidad de Talca.

Glutamate receptor-like (GLR) are non-selective, Ca^{2+} permeable ion channels regulated by amino acids. They play roles in pathogen defence, reproduction, signal transduction, and responses to biotic and abiotic stresses. Vascular plants have 20 to 70 GLR genes, making it challenging to pinpoint the specific function. In contrast, the moss *Physcomitrella patens* has only 2 GLR genes: Ppglr1 in vegetative tissues and Ppglr2 in reproductive tissues. This moss serves as a valuable experimental model due to its easy maintenance, reproduction, and short growth cycles. Studies in *A. thaliana* have linked GLR channel activity to salt stress and Ca^{2+} fluxes. The main objective was to generate genetic data involving GLR channels in salt stress response in moss. We subjected WT, Ppglr1 single mutant, and Ppglr1/2 double moss lines to salt (350 and 700 mM) and quantified growth, chlorophyll content and element accumulation (Na^+, Cl^-, K^+, Ca^{2+}). WT exhibited higher tissue survival and chlorophyll levels under salt stress compared to mutants. Element composition analysis showed an inverse relationship between K^+ and Cl^- concentrations with rising NaCl levels, and differences in WT and mutants. Altogether, those findings underscore the crucial role of GLR channels in *P. patens* physiology and their significance in salt stress resistance. Our long-term objective is to determine the mechanism of involvement of GLR in salt stress response. In Arabidopsis pollen tube, GLRs are associated with Ca^{2+}, pH and ROS signals. We started to monitor the three second messengers in moss. We present confocal microscopy showing pH and ROS gradients in WT and Ppglr1/2 cells monitored using the respective genetic probes pHluorin2 and RoGFP2. The immediate perspective of this work is to monitor the pH, Ca^{2+} and ROS signal in WT and Ppglr1/2 double moss lines under salt stress, challenging a role of GLRs in controlling those three signals.
Acknowledged: FONDECYT N1210920.

137. Characterizing β-Glucosidase from Cranberries: Evaluating Its Role in Anthocyanin Degradation through Heterologous Expression in *E. coli.* Bárbara Rivera, Marcell Gatica, Elena Amparo Uribe, Juan Román. Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile.

The American cranberry (*Vaccinium macrocarpon*) is rich in anthocyanins, secondary metabolites that offer various health benefits. However, these compounds are highly unstable, and their degradation contributes to the perishability of the fruit. Limited information exists on the enzymatic degradation of anthocyanins in cranberries. Among the few studies available, β-glucosidases have been suggested as potential enzymes involved in this process. While the gene encoding for β-glucosidase is documented in the literature, its role in anthocyanin degradation remains unclear. This project aims to confirm the presence of β-glucosidase in cranberries and determine its function in the enzymatic degradation of anthocyanins.
Using computational tools and databases, a vector with the cDNA of a potential β-glucosidase from cranberry was designed and synthesized. Heterologous expression of the designed vector in *E. coli* BL21 was carried out to obtain the enzyme, whose β-glucosidase

and anthocyanase activities were verified using pNPG and the differential pH method. Active β-glucosidase enzymes are expected to be obtained from the heterologous expression of cranberry cDNAs. The confirmation of β-glucosidase activity and its role in anthocyanin degradation will provide significant insights into the enzymatic pathways affecting anthocyanin stability in cranberries. Understanding these mechanisms can facilitate the development of targeted strategies to inhibit the enzymatic degradation of anthocyanins, thereby enhancing the shelf life and quality of cranberry products. This advancement will benefit farmers by reducing post-harvest losses and optimizing the food supply chain, leading to improved economic outcomes and consumer satisfaction.
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138. Single-molecule characterization of the effect of temperature and CspA chaperone on the TAR RNA folding mechanism. Rodrigo Rivera (rodrigo.rivera.s@ug.uchile.cl), Cayetana Zamorano, Cristian Valdevenito & Mauricio Baez. Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.

Introduction: A decrease in temperature induces the expression of specific RNA chaperones, known as cold shock proteins, which are essential for bacterial growth. This response appears to be rooted in the thermodynamic and kinetic properties of RNA molecules. Unlike protein folding, RNA folding occurs on a more rugged free energy landscape, where many alternative conformations coexist. However, temperature-dependent bulk experiments performed using traditional methodologies lack the necessary resolution to describe the folding mechanism, the misfolding distribution of RNA molecules, and how chaperones counteract this effect.
Materials and Methods: To monitor individual RNA unfolding and folding trajectories, we conducted force spectroscopy on a small RNA hairpin of 52 nucleotides between 10 and 30 C°. This approach allowed us to directly determine the misfolding probability of RNA as a function of temperature and to assess the impact of a cold shock protein, CspA, at the single-molecule level.
Results: Below 20 °C, the number of misfolding trajectories increased significantly, reaching 81 percent at 10 °C. Some of these events involved the formation of a small number of incorrect interactions that stabilized alternative RNA structures. In the presence of 1 micromolar CspA, the number of misfolding intermediates decreased; however, there was also a reduction in the number of productive folding trajectories. Additionally, CspA did not alter the unfolding barrier of the native hairpin but slowed the folding rate constant by 100 times.
Discussion: These results demonstrate that a decrease in temperature promotes the misfolding of an RNA hairpin and that CspA can reverse this effect. A molecular mechanism for CspA is proposed in this work.
Acknowledgment: FONDECYT 1231276.

139. Deciphering the Interaction Between Nitrogen Nutrition and Cellulose Metabolism in Arabidopsis thaliana Growth. Riveras E. (ejrivera@uc.cl)[1], Moreno S.[1], Nuñez V.[1], Cerda A.[1], Rothkegel K.[1], Vega A.[1], Sampathkumar A.[2], and Gutiérrez RA.[1]. [1]Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile. Millennium Institute for Integrative Biology, Millennium Institute for Center for Genome Regulation, [2]Max Planck Institute of Molecular Plant Physiology, Potsdam, Germany

Introduction: Nitrate acts as both a nutrient and a signal that regulates shoot growth through cell expansion and endoreplication. Transcriptomic data indicate that cell wall organization is overrepresented in response to nitrate. However, there is limited information on how nitrate affects the composition and properties of the cell wall during cell expansion and growth.
Methodology: To understand the effect of nitrate on cellulose metabolism, we treated plants with the cellulose biosynthesis inhibitor Isoxaben and measured shoot growth and cell expansion in nitrate-mediated growth. Additionally, we evaluated CESA velocity using spinning disk microscopy and the phosphorylation of CESA3 by Western blot analysis under contrasting nitrate conditions. We generated complemented lines by replacing the phosphorylation site with wild type, phosphomimic, and phosphonull amino acids to investigate the consequence of this phosphorylation on CESA3 velocity under contrasting nitrate conditions.
Results: Shoot growth and cell expansion in nitrate-mediated growth were reduced in plants treated with the cellulose biosynthesis inhibitor Isoxaben and in CESA complex mutant plants. We also found an increase in CESA velocity and phosphorylation of CESA3 under contrasting nitrate conditions. The phosphonull version exhibited reduced CESA3 velocity in nitrate-mediated shoot growth.
Discussion: Our results suggest that the phosphorylation of CESA3 is critical in defining its functional properties in response to nitrate, highlighting the importance of studying the interplay between nitrate and cellulose metabolism during cell expansion.
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140. Identification of the alcohol dehydrogenase (ADH) family involved in the aroma pathway of Fragaria x ananassa. Francisca Rodríguez-Arriaza[1] (franciscarodriguezarriaza@gmail.com); Felipe Moraga-Maldonado[1]; Francisca Hormazabal-Abarza[2]; Patricio Ramos[2]; Luis Morales- Quintana[1]. [1]Multidisciplinary Agroindustry Research Laboratory, Institute of Biomedical Sciences, Faculty of Health Sciences, Universidad Autónoma de Chile. Cinco Poniente #1670 Talca, Región del Maule, Chile. [2]Plant Microorganism Interaction Laboratory, Instituto de Ciencias Biológicas, Universidad de Talca, Talca, Chile.

Introduction: *Fragaria x ananassa* (commercial strawberry) is known for its organoleptic properties, such as color, texture, and aroma. The aroma is generated by the production of volatile compounds through esterification reactions between alcohols and acyl-CoAs, a process carried out by an enzyme called alcohol acyltransferase (AAT). Prior to this esterification, the enzyme alcohol dehydrogenase (ADH) converts aldehydes into alcohols. The objective of this study was to identify the isoforms of the enzyme alcohol dehydrogenase (ADH) in strawberries and to examine how their expression varies during ripening and in fruit treated with hormones after harvest.
Materials and Methodology: *Camarosa* strawberry fruits at different ripening stages were used. Additionally, different hormones, such as abscisic acid (ABA) and auxin (AUX), were applied, and their effects were evaluated at various post-application times. Bioinformatics and molecular tools were used to determine the genes and their expression.
Results: Five FaADH isoforms were identified, and computational modeling was performed, revealing the characteristic structural motifs of this enzyme family. At the molecular level, relative expression analyses showed variations in expression levels across different maturation stages, with higher expression primarily observed in early stages of maturation. Differences were also noted in response to hormonal treatments.
Conclusion: Our results suggest that FaADH isoforms play distinct roles in the biosynthesis of alcohols early in maturation, which could serve as a starting point for the biosynthetic pathway of volatile compounds.
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141. Molecular mechanisms of anthocyanin degradation in cranberries: Roles of β-glucosidase, peroxidase and poliphenol oxidase revealed by docking and UPLC-MS/MS analysis. Victoria Araya, Marcell Gatica, Elena Amparo Uribe, Juan Román (jroman@udec.cl). Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile.

Anthocyanins are bioactive pigments extensively found in the plant kingdom, contributing to the distinct colors of fruits, vegetables, and flowers. These compounds play crucial roles in plants, such as aiding pollination and providing defense mechanisms. The presence of anthocyanins is governed by the balance between their biosynthesis and degradation. While the biosynthetic pathway of anthocyanins is well-documented, the enzymatic degradation mechanisms remain less understood. Enzymes such as β-glucosidase (BGL), polyphenol oxidase (PPO) and peroxidase (POD) are suggested to be involved in anthocyanin degradation. Specifically, in plants like petunias and fruits like eggplant and *Sicilian* orange, β-glucosidase has been identified as a key enzyme in this process.
This study aimed to elucidate the molecular interactions between the anthocyanase enzyme (BGL) and the anthocyanins reported in Cranberry (*Vaccinium macrocarpon*) using molecular docking techniques, with the goal of identifying the residues likely involved in

anthocyanin interaction. Initially, the three-dimensional structures of the enzymes BGL, PPO, and POD were constructed based on their amino acid sequences, obtained through alignments within the Ericaceae family. The modeled structure of BGL, comprising 551 amino acids with a $(\beta/\alpha)_8$ barrel topology, was refined via molecular dynamics for 100 ns, yielding an average RMSD of 0.14 nm.
Molecular docking simulations revealed stable interactions between anthocyanase and the anthocyanins cyanidin 3-arabinoside and cyanidin 3-glucoside, exhibiting favorable interaction ΔG values ranging from -9.3 to -9.2 kcal/mol. Furthermore, ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS) identified fifteen anthocyanins and related compounds in cranberry samples, including cyanidin-3,5-diglucoside, cyanidin-3-galactoside, delphinidin, and procyanidin B2. This study proposes a mechanism for anthocyanin degradation and suggests strategies to enhance the stability of cranberry-based products.
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142. The Role of the N-terminal end of ECE-1c in Its Subcellular Localization in Colorectal Cancer Cells. Paula Romero Vicencio (paula.romero.1@ug.uchile.cl), María de los Ángeles Toro, Karla Villalobos-Nova, Javiera Vargas, Julio C. Tapia. Laboratorio de Transformación Celular, Programa de Biología Celular y Molecular, ICBM, Facultad de Medicina, Universidad de Chile.

Introduction: Endothelin-Converting Enzyme-1c (ECE-1c) plays a significant role in the tumor progression of various cancer types, including colorectal cancer. Several studies have shown that different post-translational modifications, such as CK2-dependent phosphorylation and ubiquitination, occurring at the cytosolic N-terminal end of the enzyme, promote both its stability and a malignant phenotype in glioblastoma, lung and colorectal cancer cells. However, it has not been evaluated how these post-translational modifications affect the subcellular localization of ECE-1c. The objective of this study is to assess how post-translational modifications occurring at the N-terminal end of ECE-1c may alter its subcellular localization in DLD-1 colorectal cancer cells.
Materials and Methods: DLD-1 cells overexpressing various phosphomimetic and phosphoresistant mutants of Flag-tagged ECE-1c, as well as a mutant resistant to proteasomal degradation, were used. The subcellular localization of the different mutants was evaluated by immunofluorescence using an antibody against the Flag tag, and images were obtained in a confocal microscope.
Results: It was observed that the localization of the enzyme was modified in the phosphomimetic and degradation-resistant mutants, compared to the phosphoresistant and wild-type proteins. The two formers were primarily located at the plasma membrane, while the two latter were situated near the Golgi apparatus.

Discussion: Post-translational modifications, specifically phosphorylation and ubiquitination, occurring at the N-terminal end of ECE-1c, modify its subcellular localization according to its protein stability.
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143. Who regulates where iron goes? Marlene Fuenzalida[1], Carlos Schomburgk[1], Pia Urbina[1], Nathalia Navarro[1], Evandro Ferrada[2], Viviana Escudero[3], Manuel González-Guerrero[3,4], Christian Dubos[5], Hannetz Roschzttardtz[1] (hroschzttardtz@bio.puc.cl). [1]Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, Chile. [2]Universidad de Valparaiso, Chile. [3]Centro de Biotecnología y Genómica de Plantas (UPM-INIA/CSIC), Universidad Politécnica de Madrid, Spain. [4]Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas. Universidad Politécnica de Madrid, Spain. [5]IPSiM, University Montpellier, CNRS, INRAE, Institut Agro, Montpellier, France.

Seeds are a primary source of dietary iron. Most of our understanding of seed iron metabolism is based on studies conducted in the model plant Arabidopsis thaliana, which has shown that iron accumulates in the vacuoles of the endodermal cell layer during seed maturation. In this study, we used Arabidopsis and maize plants as models to investigate the effect of B3 transcription factor mutations on iron distribution in seeds. Utilizing a histological method for iron detection, we determined the cellular and subcellular localization of iron in various mutant seeds. We performed an analysis using antibodies against plant ferritin and found that total ferritin levels are increased in mutant seeds, which correlates with activation of the AtFER1 promoter, indicating a repressive role of at least one of the B3 transcription factors analyzed in this study. Finally, using mutant and transgenic plant approaches, we modified iron distribution in Arabidopsis embryos. Notably, these changes did not impact total iron content in seeds, suggesting that embryonic iron distribution does not control total seed iron content.
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144. Unraveling the role of nitrate-induced endoreplication over the transcriptome and chromatin landscapes modulating leaf growth in *Arabidopsis thaliana.* Karin Rothkegel[1,2] (rothkegel.k@gmail.com), Tomás Custodio Moyano[1,3], José M. Alvarez[1,3], Rodrigo A. Gutiérrez[1,2]. [1]Millennium Institute for Integrative Biology (iBio), Santiago, Chile. [2]Center for Genome Regulation (CRG), Institute of Ecology and Biodiversity (IEB), Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile.[3]Centro de Biotecnología Vegetal, Facultad de Ciencias, Universidad Andrés Bello, Santiago, Chile.

Introduction: Understanding plant growth will provide new strategies to increase crop yield for food supply, biofuels or other applications. Nitrogen (N) is an essential macronutrient

required for plant growth. Nitrate is the main source of N in aerobic soils, and a potent signal controlling plant growth and development. Plant organ growth is defined by the interplay between cell proliferation and cell expansion. In plants, organ growth is closely linked to endoreplication, which involves DNA replication without mitosis, increasing ploidy levels and cell size.
Methods: To understand the relevance of endoreplication for nitrate-promoted leaf growth, we studied the transcriptome and chromatin accessibility of plants grown under contrasting nitrate conditions.
Results: We observed that with higher nitrate levels, the transcriptome size increases, contributed by higher ploidies. Differential expression revealed an increasing number of downregulated genes at higher ploidies, concomitant with a decreased accessibility of chromatin. Overrepresented biological processes show that “mitosis” and “regulation of cell cycle” are enriched in cells with lower ploidies and decreased at higher ploidies. Whereas “plant epidermal cell differentiation” and “cell growth” are enriched only in cells with higher ploidies.
Discussion: Our results show a dynamic transcriptome and chromatin landscape when nitrate levels are higher, suggesting that leaf cells downregulate the mitotic cell cycle to enter endocycle, allowing overexpression of necessary genes for epidermal cell growth and differentiation.
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145. Unveiling and boosting the strawberry aroma: Abscisic acid role in regulating key enzymes and volatile ester production. Darwin Sáez[1,2], Francisca Rodriguez-Arriaza[1], Felipe Moraga-Maldonado[1], Francisca Hormazabal-Abarza[1], Mariona Gil i Cortiella[3], Emilia Escalona[1], Angela Méndez-Yáñez[1], Carolina Parra-Palma[1], Patricio Ramos[4], Luis Morales-Quintana[1]. [1]Multidisciplinary Agroindustry Research Laboratory, Instituto de Ciencias Biomédicas, Facultad de Ciencias de la Salud, Universidad Autónoma de Chile. Cinco Poniente #1670. Talca, Región del Maule. Chile. [2]Programa de Doctorado en Ciencias Biomédicas, Instituto de Ciencias Biomédicas, Facultad de Ciencias de la Salud, Universidad Autónoma de Chile, Chile. [3]Multidisciplinary Agroindustry Research Laboratory, Instituto de Ciencias Aplicadas, Facultad de Ingeniería, Universidad Autónoma de Chile. Santiago, Región Metropolitana. Chile. [4]Plant Microorganism Interaction Laboratory, Instituto de Ciencias Biológicas, Universidad de Talca, Talca, Chile.

Introduction: The commercial strawberry (*Fragaria x ananassa*) is a non-climacteric fruit of great economic importance due to its food value and nutritional components such as vitamin C, folic acid, anthocyanins, flavonoids, and phenolic acids. A crucial quality factor influencing consumer acceptability is the aroma and is made of several volatile chemical compounds and is a decisive factor in fruit quality, directly influencing taste perception and consumer

acceptability, which is attributed to volatile organic compounds (VOCs), including esters, terpenes, furans, and ketones. Several enzymes are responsible for the aroma pathway, like pyruvate decarboxylase (PDC), lipoxygenase (LOX), hydroperoxide lyase (HPL), alcohol dehydrogenase (ADH) and alcohol acyltransferase (AAT), these enzymes are responsible for the transform alcohol in volatile esters. The aim of this work is exploring the changes induced by abscisic acid (ABA) hormonal treatments on the expression of *FaPDC*, *FaHPL*, *FaADH,* and *FaAAT* enzyme-coding genes, as well as their correlation with volatile ester production in strawberries.
Materials and Methods: we measure VOC by SPME-GC-MS analysis and evaluate the up and downregulated enzyme-coding genes for aroma pathway by qPCR during development and ripening of strawberry fruit.
Results: The treated fruits exhibited substantial upregulation of three *FaAAT*, *FaHPL,* and *FaPDC* genes compared to the control group. Meanwhile, the *FaADH* not change. This heightened gene expression directly correlated with increased volatile ester production, key contributors to strawberries aroma and flavor profile.
Discussion: These findings underscore the crucial role of ABA hormone in modulating the metabolic pathway of volatile esters by positively regulating *FaPDC*, *FaHPL,* and *FaAAT* genes. Beyond elucidating the molecular mechanisms underlying aromatic compound biosynthesis in fruits, this study highlights the potential of hormonal treatment as a valuable tool for enhancing the sensory attributes of agricultural products.
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146. The UDP-rhamnose/galactose transporter 4 (*Urgt4*) a key nst for biosynthesis and dimerization of rg-ii in *arabidopsis thaliana* roots. **Juan Pablo Parra-Rojas[1], Dayan Sanhueza[1], Pablo Sepulveda-Orellana[1], Sebastian Zuñiga-Pozo[1], Alvaro Miquel[1,2], Susana Saez-Aguayo[1] and Ariel Orellana[1,2] (aorellana@unab.cl). [1]Centro de Biotecnología Vegetal, Facultad de Ciencias Biológicas, Universidad Andrés Bello, Santiago, Chile. [2]Millenium Institute Center for Genome Regulation, Santiago, Chile.**

Introduction: The plant cell wall is mainly composed of polysaccharides. Among these polysaccharides, the composition and structure of pectins like Rhamnogalacturonan I and II (RG-I and RG-II) are important for the size and shape of the primary root in plants. Rhamnose is a key monosaccharide for RG-I and RG-II biosynthesis in the Golgi lumen. Rhamnose is provided by UDP-Rhamnose, and this nucleotide sugar is synthesized in the cytoplasm. The incorporation of UDP-Rhamnose into the Golgi is carried out by nucleotide sugar transporters called URGTs. Arabidopsis has six URGT genes, and to date, there is no evidence of size and shape consequences associated with the lack of some URGTs on root development.

Materials and Methods: Expression analysis by RT-qPCR and phenotype screening in single URGT mutants were performed to associate a URGT member with proper root growth. Biochemical, electrophoretic, and immunofluorescent methods were employed in wild-type and mutant roots to compare RG-I and RG-II pectins.
Results: URGT4 is highly expressed in seven-day-old roots, and molecular rescue lines show a restored wild-type phenotype. The *urgt4-2* mutant exhibits slight differences in monosaccharide composition in roots, suggesting effects on RG-II. Immunolabeling against arabinan, branched-galactan, and the RG-I backbone suggests minor effects on RG-I pectin. On the other hand, PAGE analysis shows monomers and dimers of RG-II in mutant roots, suggesting an altered structure *in muro*. Furthermore, the mutant supplemented with boric acid in the culture medium shows a partial restoration of the wild-type phenotype and exhibits a decrease in the presence of RG-II monomers
Discussion: Our results suggest that URGT4, among others URGTs, is the key player in root development, maintaining the correct RG-II structure.
Acknowledgment: ANID – Millennium Science Initiative Program – ICN2021_044; FONDECYT 1230859; Mizutani Foundation for Glycoscience.

147. LncRNA KCNQ1OT1 of peripheral blood mononuclear cells: Potential biomarker for lipid-lowering response to atorvastatin in hypercholesterolemic patients.
Humberto Vélez, Pía Loren & Luis A. Salazar (luis.salazar@ufrontera.cl). Universidad de La Frontera, Temuco, Chile.

Atorvastatin is extensively used to treat hypercholesterolemia (HC). However, the wide interindividual variability observed in response to this drug still needs further elucidation. Recent studies have reported the role of long non-coding RNAs (lncRNAs) in the metabolism of lipids. Among this, KCNQ1OT1, the KCNQ1 opposite strand/antisense transcript 1, is capable of interacting with miRNAs, RNAs and proteins, thereby affecting gene expression and various cell functions. In addition, KCNQ1OT1 is dysregulated in a wide range of diseases, and it is speculated to act as a therapeutic target for treating various human diseases. Moreover, it was demonstrated that KCNQ1OT1 promotes macrophage lipid accumulation and accelerates the development of atherosclerosis. Nevertheless, there are no studies to date that show their role in the response to treatment with statins. Thus, the aim of this study was to assess the levels of expression of lncRNA KCNQ1OT1 in leukocyte cells of patients with HC after treatment with atorvastatin. Twenty hypercholesterolemic patients were treated for four weeks with atorvastatin (20 mg/day). The lipid profile was determined before and after drug administration using conventional assays. The expression of lncRNA KCNQ1OT1 was assessed in peripheral blood cells by RT-qPCR. As expected, atorvastatin improved the lipid profile, decreasing total cholesterol, LDL-C, and the TC/HDL-C ratio ($p < 0.0001$). Atorvastatin had a negative regulatory effect on the expression of lncRNA KCNQ1OT1 ($p=0.022$) in HC subjects after treatment. Our findings show that the lncRNA KCNQ1OT1 expression in HC patients play a role in the variability in the lipid-

lowering response to atorvastatin. Further research is needed to clarify the biological impact of KCNQ1OT1 on cholesterol homeostasis and treatment with statins.
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148. The intracellular processing of mollusk hemocyanins from *Concholepas concholepas* (CCH) and *Megathura crenulata* (KLH) modulates their proinflammatory effects in murine dendritic cells. Michelle L. Salazar[1], Javier Bustamante[1], Claudia d'Alencon[1], Diego Díaz-Dinamarca[1], Augusto Manubens[1,2], Fabián Salazar[1, 3], María Inés Becker[1,2]. [1]Laboratorio de Inmunología, Fundación Ciencia y Tecnología para el Desarrollo (FUCITED). [2]Laboratorio de Investigación y Desarrollo, Biosonda S.A. [3]Medical Research Council Centre for Medical Mycology, University of Exeter, Exeter, United Kingdom.

Mollusk hemocyanins are widely used in biomedicine as adjuvants, carriers, and immunostimulants because they promote Th1 immunity. Hemocyanins have a high molecular mass (8 MDa), complex structure, and diverse glycosylations. They are multiligands of innate immune receptors like Toll-like receptor 4 and mannose receptor on antigen-presenting cells (APCs). These receptors regulate the proinflammatory response, endocytosis, and intracellular processing via proteasomal and lysosomal pathways, generating peptides loaded onto class I and II major histocompatibility complexes. Unlike model antigens, such as Ovalbumin (OVA, 45 kDa), APCs slowly process hemocyanins, resulting in a delayed proinflammatory response. Here, we aim to explore the main intracellular proteolytic pathways involved in hemocyanin processing and proinflammatory effects.We hypothesize that: "Hemocyanins are processed through both proteasomal and lysosomal pathways, modulating the proinflammatory response towards Th1 polarization".
We used the JAWS-II cell line and bone marrow-derived dendritic cells as murine APCs, which were stimulated with native KLH and CCH hemocyanins. Confocal microscopy showed that CCH and KLH co-localized with lysosomal compartments (LAMP1) after 96 hours, whereas OVA co-localized after 24 hours. Ongoing analyses focus on co-localization with proteasomal proteins. Flow cytometry has confirmed that co-localization timing correlates with peak levels of IL-6, IL-12p40, IL-17, IL-2, and costimulatory molecules (CD80 and CD86), with earlier TNF secretion observed. Cytokine secretion peaked at 24 hours when partially digested hemocyanins were used. Ongoing immunofluorescences will characterize the proteolytic activity of proteasomes and lysosomes. These findings confirm the critical role of hemocyanin lysosomal and proteasomal processing in their delayed immunostimulatory effects on APCs.
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149. Exploring new plant models for study in biotechnology. The highly tolerant Antarctic moss, *Sanionia uncinata* and its contribution to understand the tolerance to abiotic stress. Alex SanMartin-Davison (alexsanmartin@utalca.cl), Oscar Arrey-Salas, Monica Yañez. Erwan Michard

When studying molecular events related to abiotic stress tolerance in plants, models such as *Arabidopsis thaliana, Solanum lycopersicum, Nicotiana tabacum* and *Oryza sativa* are commonly used. However, in all these models, the traits controlling abiotic stress tolerance are regulated by multigene families, which complicate the use of strategies such as mutation or silencing due to gene redundancy. Thus, plant species with a simpler genomic composition, such as mosses, may be useful for understanding these mechanisms. *Physcomitrium patens* is the most used moss, but it is sensitive to abiotic stress. Here we present the Antarctic moss *Sanionia uncinata*, noted for its tolerance to UV radiation, dehydration, salinity, and darkness, as a potential model for studying abiotic stress. Finally, our evidence supporting the potential use of *S. uncinata* as a model moss species in relation to the study of abiotic stress.
Moss pieces from the Antarctic Peninsula (Fildes Bay) were kindly obtained from the laboratory of Dr. Angelica Casanova-Katny. Moss was rehydrated, after 2 years of storage at 4°C, in distilled water for 2 months until the gametophores emission (17°C, photoperiod 16/8, 55 umol/m-2*S-2. From this tissue we propagated in perilite:vermiculite:peat: 1:1:3, for temperature (max 28°C) and salt (350mM NaCl) treatments. Tissue was also disinfected and introduced under in-vitro conditions. Vegetatively propagated tissue was used for protonema induction and protoplasts were obtained for transient transformation experiments with the gCaMP7C probe.
We propose using *Sanionia* as an experimental system to study genes involved in abiotic stress response. Such study may participate in understanding the unique adaptability of the Antarctic moss adapted to survive in extreme conditions.
Acknowledgment: FONDECYT-1210920.

150. Biosynthesis of ternary quantum-dots for photovoltaic solar cells. Sánchez, Natalia (nasanchez2017@udec.cl) and Martínez-Oyanedel, José. Laboratorio de Biofísica Molecular, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.

The current global energy crisis has led to the search for non-conventional, efficient and sustainable renewable energy sources. One of the alternatives is quantum-dot sensitized photovoltaic solar cells (QDSSC) that use the solar energy to produce electricity.
Quantum-dots (QD) are fluorescent semiconductor nanoparticles synthesized by environmental microorganisms, QD have optical and electrical properties suitable for use in the construction of QDSSCs that are more efficient than their dye-sensitized counterparts, DSSCs.
For this research, from a library of 65 strains of heavy metal-resistant environmental bacteria, seven strains were able to produce binary QDs in the presence of cysteine as a sulfur source and cadmium chloride ($CdCl_2$), zinc chloride ($ZnCl_2$), or copper chloride ($CuCl_2$)

as a second metal. We optimized the conditions for production of binary QDs using different culture medium and buffers.
From these, we realized the synthesis of ternary QDs by means of cation exchange by adding silver nitrate ($AgNO_3$) to the binary QDs. The best conditions for the synthesis of ternary QDs were by performing the synthesis in the absence of light and sub lethal $AgNO_3$ concentrations.
We characterized the binary and ternary QDs in their fluorescence spectra and presence of the components by X-ray fluorescence. The ternary QTs are being purified to assemble QDSSC cells to evaluate the efficiency in electricity production.
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151. Extracellular vesicles from MDA-MB-231 breast cancer cells enhance endothelial cell tube formation in a Caveolin-1-dependent manner. Sofía Sanhueza[1,2,3] (sofiasanhueza@ug.uchile.cl), América Campos[1,3], Renato Burgos[1,3], Diego Peña[1,3], Baohai Shao[4], Jay Heinecke[4], Mariana Cifuentes[2,3], Lisette Leyton[1,3], Andrew F.G. Quest[1,3]. [1]Laboratory of Cellular Communication, Center for Studies on Exercise, Metabolism, and Cancer (CEMC), Faculty of Medicine, Universidad de Chile, Santiago, Chile. [2]Laboratory of Obesity and Metabolism in Geriatrics and Adults (OMEGA), Institute of Nutrition and Food Technology (INTA), Universidad de Chile, Santiago, Chile. [3]Advanced Center for Chronic Diseases (ACCDiS), Universidad de Chile, Santiago, Chile. [4]Division of Metabolism, Endocrinology and Nutrition, University of Washington, Seattle, USA.

Introduction: Breast cancer is the leading cause of cancer-related death in women worldwide. During tumor development, cancer cells acquire several biological characteristics, including increased migration and invasion, as well as the ability to induce angiogenesis. Interestingly, many of these characteristics are enhanced by the expression of Caveolin-1 (CAV1), a multifunctional membrane protein that is typically upregulated in the final stages of cancer. Additionally, extracellular vesicles (EVs) released by CAV1-expressing MDA-MB-231 breast cancer cells promote migration and invasion of less aggressive recipient cells. However, it remains unknown whether CAV1-containing EVs released from breast cancer cells favor angiogenesis-related properties in endothelial cells in a CAV1-dependent manner.
Methods: EVs were isolated from conditioned media of MDA-MB-231 (WT), MDA-MB-231 (shC; short hairpin control), and MDA-MB-231 (shCAV1; short hairpin CAV1). EVs were characterized using Nanoparticle Tracking Analysis (NTA) and Western blotting. The protein content of these EVs was further analyzed using shotgun proteomics. The effect of CAV1-containing EVs on Ea.hy926 endothelial cells was evaluated using the endothelial cell tube formation assay.
Results: NTA revealed that the mean particle size ranged from 120 to 180 nm. CAV1 was detected in MDA-MB-231 WT and shC EVs but not in MDA-MB-231 shCAV1 EVs. Proteomic analysis revealed the presence of CAV1 and angiogenic-related proteins, such as Tenascin-

C, only in WT and shC but not in shCAV1 EVs. Importantly, CAV1-containing EVs enhance endothelial cell tube formation in Ea.hy926 cells.
Discussion: These results implicate CAV1 in modulating the content of EVs liberated by MDA-MB-231 breast cancer cells to promote endothelial cell tube formation. Therefore, understanding the mechanisms by which CAV1-containing EVs promote angiogenesis-related responses likely represents a fruitful area of research with great therapeutic potential.
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152. Dysregulation of zinc transporters in cancer progression: Exploring differential expression patterns in prostate and gastric cancer. María Fernanda Segovia (maría.segovia@alumnos.ucn.cl)[1], Samantha Acevedo[1], Rodrigo Calderón[1]and Erwin de la Fuente-Ortega [1,2,3]. [1]Laboratorio de Estrés Celular y Enfermedades Crónicas no Transmisibles, Universidad Católica del Norte, Coquimbo 1781421, Chile. [2]Núcleo de Investigación en Prevención y Tratamiento de Enfermedades Crónicas no Transmisibles (NiPTEC), Universidad Católica del Norte, Coquimbo 1781421, Chile. [3]Centro de Investigación y Desarrollo Tecnológico en Algas y Otros Recursos Biológicos (CIDTA), Facultad de Ciencias del Mar, Universidad Católica del Norte, Coquimbo 1781421,Chile.

Introduction: Dysregulation of the zinc transporters ZIP and ZnT could significantly impact the progression of prostate and gastric cancer. Prostate cancer studies show tumors often exhibit a Down/Up pattern of ZIP and ZnT expression, respectively. However, genetic variability suggests other patterns may exist in this cancer. In contrast, less is known about zinc transporter dysregulationin gastric cancer, necessitating cellular models to investigate its effects in this cancer.
Materials and Methods: For prostate cancer, we explored new patterns of zinc transporter dysregulation by analyzing available mRNA databases in GEO-NCBI (GSE11836). For gastric cancer, we used AGS cancer cells and normal GES-1 cells. We assessed cell viability to $ZnCl_2$ exposure (0-1000 µM) via MTS assay, zinc transporter mRNA expression by RT-qPCR, and examined intracellular zinc distribution with the Fluozin 3-AM sensor.
Results: In silico analysis revealed a new ZIP-Up/ZnT-Down pattern in some prostate tumors. In gastric cancer, AGS cells tolerated zinc better (IC50 301.7 µM) than GES-1 (IC50 158.2 µM). AGScells exhibited higher ZIP (ZIP4, ZIP8) and ZnT (ZnT4, ZnT5, ZnT6) expression, while ZnT7 expression was lower. Fluozin 3-AM labeling indicated higher intracellular zinc in AGS cells compared to GES-1 cells after 24 hours of zinc incubation.
Discussion: Different zinc transporter patterns in prostate cancer suggest varying mechanisms that potentially induce epithelial-mesenchymal transition. AGS cells´ higher zinc tolerance and the ZIP- Up/ZnT-Up pattern, correlated with increased intracellular zinc levels, indicate possible adaptive mechanisms in cancer cells. These findings highlight

distinct zinc transporter dysregulation patternsin prostate and gastric cancers and suggest areas for future research to understand their role in cancerprogression.
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153. Insertion of *Medicago sativa* as a sustainable strategy for the management of Cauquenes tailing. Gladis Serrano [1,2](gladis.serrano@postgrado.uoh.cl), Humberto Aponte[3,4], Mauricio Latorre [1,2,4,5]. [1]Laboratorio de Bioingeniería; Instituto de ciencias de la ingeniería; Universidad de O'Higgins, Rancagua, Chile. [2]Centro de biología de sistemas para el estudio de comunidades extremófilas de relaves mineros (SYSTEMIX), Universidad de O'Higgins, Rancagua, Chile. [3]Laboratorio de Ecología Microbiana del Suelo y Biogeoquímica, Instituto de Ciencias Agroalimentarias, Animales y Ambientales (ICA3), Universidad de O'Higgins, San Fernando, Chile. [4]Centro de Biología de Sistemas para la Sanidad Vegetal (BioSaV), Instituto de Ciencias Agroalimentarias, Animales y Ambientales (ICA3), Universidad de O'Higgins, San Fernando, Chile. [5]Laboratorio de bioinformática y expresión génica, INTA, Universidad de Chile, Santiago, Chile.

Introduction: Chile, a world leader in copper production, faces the challenge of managing a vast number of mining waste deposits, known as tailings. Of the 764 existing tailings, 85% are inactive or abandoned. The growing concern about these environmental liabilities focuses on transitioning towards a circular economy, aiming to convert these wastes into economic assets. In this context, an example is the Cauquenes tailing in the O'Higgins region, currently exploited by Minera Valle Central for the extraction of copper and molybdenum. However, this extraction does not compromise the chemical and physical stability of the tailing. Therefore, this project proposes to analyze the conditions under which *Medicago sativa* (Alfalfa) can develop in the Cauquenes tailing for phytostabilization purposes.
Materials and Methods: The conditions of the tailing were evaluated through physicochemical analyses and studies of microbiological metabolic functions, as well as plant development studies. The following conditions were used: 1) 100% tailing; 2) 2.5% compost; 3) 5% compost; 4) 10% compost, along with an external control using agricultural soil. Three commercial varieties of alfalfa were used to increase the chances of growth in the tailing.
Results: Based on physiological parameters, a variety of alfalfa corresponding to the "superlechera" variety was selected, which achieved a similar development to the agricultural control with the use of 10% compost.
Discussion: This could provide a sustainable scenario for the cultivation of other species with potential commercial applications or other purposes, achieving sustainable soil use in the medium term.

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154. N6-methyladenosine (m6A) epitranscriptomic changes induced by chronic stress in neuropile stratum of CA1 region in rat hippocampus. J.P. Silva[1-2] (juan.silva.r@ug.uchile.cl), W.A. Corrales[1-2], J. Catalán-Casanellas[1], Olave F.A.[1], V. Maracaja-Coutinho[2] y J.L.Fiedler[1]. [1]Laboratory of Neuroplasticity and Neurogenetics, Department of Biochemistry and Molecular Biology. Faculty of Chemical and Pharmaceutical Sciences Universidad de Chile, Santiago, Chile. [2]Laboratory of Integrative Bioinformatics, Advanced Genomic Unit – UGA & Center of Molecular Modeling, Biophysics and Bioinformatics – CM2B2 and Advanced Center for Chronic Diseases ACCDiS, Faculty of Chemical and Pharmaceutical Sciences Universidad de Chile, Santiago, Chile.

Introduction: Major depressive disorder is a neuropsychiatric disorder associated with cognitive decline and behavioral impairment. In rat models of chronic restraint stress (CRS), cognitive decline on spatial memory differs depending on sex and it is attributable to transcriptional and morphological changes on hippocampal neurons from CA1 region. Recent studies evidence that stress can trigger N6-methyladenosine (m^6A) modification on total brain RNAs from mice depending on the brain region and have behavioral effects. Nevertheless, specific changes at single molecule and per base levels have not been reported. Neurons have specific compartmentalization mechanisms to transport mRNAs from Soma to Dendrites at synaptic sites to increase the efficiency of neuronal communication. However, destination and local translation signals for transcripts are still unclear and literature suggests that they might be related to RNA modifications and protein interactions.
Materials and Methods In this work, we employed a direct-RNA sequencing approach with Nanopore technology to identify m^6A modifications in mRNA derived from stratum-dissected pooled samples (n=3, neuropile stratum) from CA1 hippocampal region of CRS female rats.
Results: m^6A-aware basecalling by ModPhred/*m6ABasecaller* software revealed 1,111 m^6A marks in the CRS condition while controls present 1,142 sites. Deepening into the data, we identified 665 common m^6A modifications (42%), while 30% were removed by the stressful stimulus and 27,7% were induced by stress. Enrichment analysis pinpoints that such modified transcripts were related to catabolic processing of proteins and vesicle secretion.
Discussion: This is the first description of the chronic stress effects on neuropile local epitranscrptome at individual molecule resolution, opening the window for *in silico* prediction of protein interactions and destination of transcripts at synaptic sites, shedding light to the morphological and behavioral changes.
Acknowledgement: Beca Doctorado Nacional ANID N°21220964 (JPS), FONDECYT Regular 1230471 (JLF) and 1211731 (VMC), FONDAP 15130011 and 1211731 (VMC).

155. Analysis of the effect of nanobubbles in the growth rate of Lemna sp. (duckweed).
Jairo Tapia Tejos[1,2] (jairotapiatejos@gmail.com), Victoria Flores Del Pino[1,2], Adrian A. Moreno[2], Fernando D. Gonzalez[1]. [1]Center of Bioinformatics and Integrative Biology (CBIB), Facultad de Ciencias de la Vida, UNAB. [2]Centro de Biotecnología Vegetal (CBV), Facultad de Ciencias de la Vida, UNAB.

Introduction: *Lemna sp.* (Duckweed) is a family of aquatic plants known for being the fastest-growing angiosperms and also for their easy of handling in the laboratory. Previous studies analyzing certain species of the *Lemna* genus (Wolffia sp.) have demonstrated their potential use as food due to their high protein content, suggesting the capacity of *Lemna sp.* to be used as a food source and to become a candidate model for developing food alternatives beyond its current use as animal fodder. Evidence from previous laboratory work reported that supplemented N-medium with gas increase the growth rate of other angiosperms like *Arabidopsis thaliana*, providing the basis for this research.
Objective: Analyze the effect of gas in the growth rate of *Lemna sp.*
Materials and Methods: A cultivation protocol for *Lemna* in the presence of gases nanobubbles was developed. Growth parameters and the metabolic impact of the treatment were evaluated based on the effects on fresh and dry biomass, as well as total protein content and changes on gene expression.
Results: A significant increase in biomass was observed in plants treated with the nanobubbles. Additionally, an increase in total protein content was demonstrated.
Discussion: The results suggest that nanobubbles have a beneficial effect on nutrient absorption by the plant, enhancing its growth rate, biomass accumulation and total protein production.
Acknowledgment: FONDEF ID22I10344.

156. Artificial Protein Design: Fulfilling FAO Criteria for Essential Amino Acids.
Dory Toledo[1] (dotoledo2018@udec.cl), Amparo Uribe[2], José Martínez-Oyanedel[1], Maximiliano Figueroa[1]. [1]Laboratorio de Biofísica Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción. [2]Laboratorio de Enzimología, Facultad de Ciencias Biológicas, Universidad de Concepción.

Introduction: Proteins are crucial for human nutrition, especially for high-performance athletes and individuals with selective diets, such as vegetarians and vegans. However, current protein sources often fail to provide essential amino acids in the appropriate proportions. This study proposes synthesizing an artificial protein that meets the quality criteria established by the FAO. We hypothesize that this artificial protein can fulfill the essential amino acid requirements and be utilized as a dietary supplement in the food industry.
Materials and Methods: The primary objective was to create a protein easily digestible by human enzymes. A combinatorial process was used to design the sequence based on the

required amino acid proportions. Bioinformatics tools verified digestibility and modeled the protein. After designing the gene (cloned into vector pET-15), the protein was produced, but its initial low molecular weight posed challenges in expression. To address this, molecular biology tools were used to duplicate the protein sequence. The insert was subcloned into the same vector, and restriction analysis confirmed the new plasmid's status. Bacterial transformation in *E. coli* BL21 (DE3) was then performed for expression and purification.
Results: Preliminary results showed successful vector-instert ligation, with restriction analysis confirming the correct double insert in the vector. These results validated the creation and maintenance of the desired protein sequence to be expressed in bacteria.
Discussion: Current efforts focus on optimizing the expression of this duplicated protein. Initial findings demonstrate the feasibility of synthesizing an artificial protein that meets FAO criteria for essential amino acids. Further work will refine the expression process and evaluate practical applications in various dietary contexts. This research has the potential to significantly impact the food industry by providing a high-quality protein supplement suitable for diverse dietary needs.
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157. Higher stability of Endothelin-converting enzyme 1-c promotes chemoresistance and invasion in non-small cell lung cancer cells. María de los Ángeles Toro[1] (maria.toro.b@ug.uchile.cl), Karla Villalobos-Nova[1], Cristopher Almarza[1], Paula Romero Vicencio[1], Javiera Vargas[1], Ignacio Niechi[2], Julio C. Tapia[1]. [1]Laboratorio de Transformación Celular, Instituto de Ciencias Biomédicas (ICBM), Facultad de Medicina, Universidad de Chile. [2]Laboratorio de Biotecnología tumoral, Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile

Introduction: The Endothelin-Converting Enzyme-1 isoform c (ECE-1c) is important in cancer progression and its N-terminal domain possess a conserved Lys-6 residue. Interestingly, ECE-1c with a Lys-6 mutated to arginine (ECE-1c^{K6R}) is highly stable when expressed in colorectal cancer and glioblastoma cells, but also promotes cancer stem cells traits and hence an aggressive phenotype, such as increased chemoresistance and invasiveness. However, the effect of ECE-1c^{K6R} on those traits in non-small cell lung cancer cells is unknown. Thus, our aim was to evaluate the effect of ECE-1c^{K6R} on cisplatin-resistance and invasion in H1299 lung cancer cells.
Materials and Methods: Mock (empty vector), wild-type (ECE-1c^{WT}) and highly stable (ECE-1c^{K6R}) expressing clones were developed in H1299 lung cancer cells. Transcript and protein levels of stemness genes related to chemoresistance and invasion were measured by RT-qPCR and western blot, respectively. Cisplatin-resistance was evaluated by MTS assay and invasion evaluated on Matrigel chambers.
Results: H1299 cells expressing ECE-1c^{K6R} displayed high levels of mRNA and proteins of genes related to chemoresistance and invasion, compared to mock cells. Cisplatin-

resistance and invasion were improved in H1299 cells expressing ECE-1c^{K6R} in comparison to mock cells.
Discussion: A highly increased stability of ECE-1c promotes aggressiveness traits in non-small cell lung cancer cells, such as increased mRNA and protein levels of stemness genes, as well as cisplatin-resistance and invasion. Thus, the highly stable ECE-1c protein may be considered a novel poor prognostic biomarker in lung cancer patients.
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158. Study of the structural determinants of the modulation of the TRPM4 channel by 9-phenanthrol as an anti-hypertensive potential. Alonso Torres (a.torresoso70@gmail.com), Maximiliano Rojas, Ignacio Díaz-Franulic, Valeria Márquez-Miranda, Fernando Danilo González-Nilo. Center for Bioinformatics and Integrative Biology, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago, Chile

Introduction: Transient receptor potential melastatin subfamily member 4 (TRPM4) is a calcium-activated, monovalent-selective cation channel impermeable to calcium, it has been associated with hypertension due to its predominant expression in vascular smooth muscle cells and the heart and role on the resting membrane potential. Several preclinical in vivo pharmacological studies use the combination of heterologous mouse models expressing the human TRPM4 channel and his highly specific inhibitor, 9-Phenantrol, whose binding site remains unknown. Recent studies have reported different species-specific responses of human and mouse channels and the lack of information on the binding site of 9-phenantol can lead to misinterpretations. In this study we aim to identify the specific regions of TRPM4 influenced by 9-phenanthrol to define the molecular mechanisms governing its activation and inactivation pathways in human and mouse models.
Materials and Methods: The open and closed TRPM4 structures of both species were described as well as identified the active site and associated docking through the Schrodinger suite. These systems were prepared for the CHARMM36 force field using the CHARMM-GUI suite following a 1 μs simulation with the AMBER software, and further channel closing analysis through HOLE suite.
Results: Three potential binding sites were assessed with significant hydrophobic and charged interactions with 9-phenantrol. In addition, 9-phenantrol binds to a hydrophobic pocket sited on the transmembrane domain of TRPM4 where calcium also binds for channel opening. The binding to this pocket lead to the closing of the channel observed 1 μs after its addition.
Discussion: As of now, these exploratory experiments demonstrate a promising binding site for 9-phenantrol to interact and modulate TRPM4, being the first steps toward a detail characterization of them.
Acknowledgment: FONDECYT 1221498, FONDECYT 11241081.

159. Enhancing biomass production of *Enterococcus faecalis* in Bioreactors. Jorge Torres Robles[1,2] (jorge.torres@postgrado.uoh.cl), Mauricio Latorre Mora[1,2,3]. [1]Laboratorio de Bioingeniería; Instituto de ciencias de la ingeniería; Universidad de O'Higgins, Rancagua, Chile. [2]Centro de biología de sistemas para el estudio de comunidades extremófilas de relaves mineros (SYSTEMIX), Universidad de O'Higgins, Rancagua, Chile. [3]Laboratorio de bioinformática y expresión génica, INTA, Universidad de Chile, Santiago, Chile.

Introduction: *Enterococcus faecalis*, a gram-positive bacterium, is a valuable model for studying pathogenic bacteria in bioreactors. Its use in laboratory and bioreactor settings helps researchers understand bacterial behavior, virulence, and drug resistance, providing insights into pathogen detection, virulence factor regulation, and vaccine development.
Materials and Methods: Technical requirements for bioreactor management, including gas supply, cooling systems, and sterility, were analyzed using a Minifors 2 bioreactor (Infors Switzerland). To understand abiotic and biotic factors affecting biomass production, E. faecalis was selected for a model bioreactor growth study. The 3L bench-top bioreactor in N medium (Peptone 10 g/L, Yeast extract 5 g/L and, 10 g/L Na_2HPO_4) (Minifors 2, Infors HT Switzerland), controlled by EVE® software, was used. Sensors monitored foam, temperature, pH, pO2, and agitation speed. Optimal biomass production conditions reported in literature were replicated.
Results: *E. faecalis* was cultured in N medium with varying glucose concentrations (0-10%), achieving the highest rate of biomass production at 0.5% glucose (1.398 ± 0.112 h^{-1}). Glucose consumption rates were measured, and a Monod growth equation was modeled. Pre-inoculum cultures were incubated and transferred to a fermenter with 0.6 L sterile N medium. The highest biomass concentration of 17 g/L was achieved at 8 hours.
Discussion: These results indicate that *E. faecalis* can be effectively scaled up in bioreactors, optimizing biomass production under controlled conditions. Future work aims to implement a fed-batch culture scheme with glucose feed under optimal conditions, maintaining pH 7, 37°C, and 20% dissolved oxygen. Critical bioprocess parameters such as mass transfer rate, oxygen absorption rate, and oxygen transfer rate were calculated using EVE software, providing valuable insights for future bioprocess optimization.
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160. Cardiomyocyte VCAM-1 prevents cardiac dysfunction induced by a high-fat diet. Mayarling F. Troncoso[1,2] (mayarlingtroncosom@uchile.cl), Laura Navarrete-Gallegos[1], Jafet Ortiz-Quintero[1], Felipe Muñoz-Cordova[1], Danica Jimenez-Gallegos[1], Ximena Calle-Chalco[1], Francisco Pino de la Fuente[1], Fernanda Sanhueza-Olivares[1], Angélica Ortega-Muñoz[1], Claudia Muñoz[1], Alejandra Guerrero-Moncayo[1], David Silva[1], Elsa Rocío Bascuñán[1], Magda C. Díaz-Vesga[1], Fernanda Zapata-Neweu[1], Brenda Becerra-Leiva[1], Mario Chiong[1], Anwarul Ferdous[4], Joseph A. Hill[4], Sergio Lavandero[1,4]. [1]Advanced Center for Chronic Diseases

(ACCDiS), Faculty of Chemical and Pharmaceutical Science & Faculty of Medicine, University of Chile, Santiago. [2]Department of Medical Technology, Faculty of Medicine, University of Chile. Santiago. [3]Cardiovascular Diseases Division, Faculty of Medicine, Pontifical Catholic University of Chile, Santiago. [4]Cardiology Division, University of Texas Southwestern Medical Center, Dallas.

Introduction: Obesity-induced cardiomyopathy is a clinical condition with detrimental effects on myocardial function, but the molecular mechanisms are not thoroughly studied. Vascular cell adhesion protein-1 (VCAM-1) is a pro-inflammatory protein canonically involved in the transmigration of inflammatory cells. We aimed to investigate the role of cardiac VCAM-1 in an experimental obesity-induced cardiomyopathy model using cardiomyocyte-VCAM-1-knockout mice (cVCAM-1-KO).
Methods: C57BL/6N cVCAM-1 KO and wild type f/f male mice were fed with control or high-fat diet (HFD) for 25 weeks (Bioethical protocol CBE2020-04, U.Chile). We evaluated body, adipose and heart weight, cardiomyocyte cross-sectional area (CSA), fibrosis, and echocardiography was performed exercise and glucose tolerance tests, the circulating levels of insulin and soluble VCAM-1 (sVCAM-1) by ELISA, mRNA levels of ANP, BNP, and β-MHC by RT-qPCR and Immuno-blot for VCAM-1 and pAKT(Ser473), and immunohistochemistry for VCAM-1. Statistical analysis: mean±SD was used in n=3±15 and analyzed by non-parametric one-way ANOVA. $p<0.05$ was considered significant.
Results: Mice fed with HFD developed obesity-induced cardiomyopathy. The cVCAM-1-KO mice fed with HFD developed diastolic and systolic dysfunction, exercise intolerance, pathological cardiac hypertrophy (heart weight/body weight, CSA, and mRNA expression of ANP and BNP), and fibrosis compared with the controls (f/f HFD mice). Both HFD groups presented glucose intolerance. However, hyperinsulinemia was only increased in f/f HFD mice. Also, cVCAM-1-KO HFD mice showed increased pAKT(Ser 473) under an insulin pulse. sVCAM-1 increased in f/f HFD animals but not in cVCAM-1-KO HFD.
Discussion: c-VCAM-1-KO mice fed with HFD developed obesity-induced cardiomyopathy and exacerbated the hypertrophic phenotype, suggesting that cardiomyocyte VCAM-1 has a protective role.
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161. Evaluating the role of oxidized manganese species on amyloid formation. Diego Troncoso Alarcon (diego.troncoso.a@usach.cl), Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile.

Introduction: Amyloids are highly stable aggregates characterized by intermolecular contacts and a cross-beta sheet motif. Some amyloids are pathological, but the great majority fulfill many physiological functions in all kingdoms of life, participating in structure, storage, and signaling. Different studies have shown that certain amyloids can act as

enzyme-like catalysts. We previously reported that a small peptide (sequence SDIDVFI) can self-assemble into amyloids in the presence of manganese ions (Mn^{+2}), which specifically catalyze the hydrolysis of adenosine triphosphate (ATP) into adenosine diphosphate (ADP) and monophosphate (AMP). The aim of this work was to determine the role of oxygen and pH-mediated oxidation of Mn^{2+} in the aggregation of SDIDVFI.
Materials and Methods: Peptide SDIDVFI with acetylated and amidated NH_3 and COOH terminals was exposed to different pH conditions and manganese chloride ($MnCl_2$) concentrations in presence and absence of oxygen, to evaluate how the formation of oxidized manganese species affects peptide self-assembly into amyloids. Aggregation and oxidation were followed by colorimetry, fluorescence, and transmission electron microscopy (TEM).
Results: The formation of oxidized manganese was visualized by a brown coloration that formed with and without the peptide. The formation of these oxidized species is dependent on both a basic pH (8.0 and above) and the presence of oxygen. The presence of nanoparticles approximately 50 nm in size were detected as part of the oxidized species. Aggregation assays with the amyloid-specific fluorescence probe thioflavin T (Th-T) showed that oxidized manganese appears not to affect nor to be required for the self-assembly of SDIDVFI into amyloids.
Conclusions: During the assembly of peptide SDIDVFI in presence of Mn^{2+}, oxidized manganese species form in a pH- and oxygen-dependent manner. These species are not required for amyloid formation.
Acknowledgment: FONDECYT 1211821.

162. Transcriptome-guided assembly for coding and non-coding RNAs in cardiac hypertrophy induced by norepinephrine. Sebastián Urquiza-Zurich[1](sebastian.urquiza@ug.uchile.cl), Evelia Coss-Navarrete[2], Allan Peñaloza-Otárola[1], Paulo P. Amaral[3], Sergio Lavandero[1,4], Vinicius Marcaja-Countino[1]. [1]Advanced Center for Chronic Diseases (ACCDiS), Chile. [2]Universidad Nacional Autónoma de México (UNAM), México. [3]Insper Instituto de Ensino e Pesquisa, São Paulo, Brazil. [4]Cardiology Division, University of Texas Southwestern Medical Center, Dallas, USA.

Background: Pathological cardiac hypertrophy (PCH) is a response to pressure overload and neurohumoral dysregulation, leading to irreversible cardiomyocyte enlargement by reactivating the expression of the fetal gene program. However, the molecular mechanisms in the genesis and development of PCH remain not entirely understood. We proposed here that some lncRNAs, a type of non-coding transcript, play a critical role in the development of catecholamine-induced PCH.
Methods: We analyzed an RNA-seq dataset in neonatal rat ventricular myocytes (NRVMs) treated with norepinephrine (NE). We set up a transcriptional guided assembly pipeline using Stringtie, coupled with coding potential algorithms (RNAmining, CPAT and CPC2) and Strict Mode pipeline for characterizing novel coding and non-coding transcripts, such as lncRNAs.

Then, we performed differential expression analysis with DESeq2, filtered differentially expressed genes (DEGs) by |log2[fold change]| ≥ 1, and p adjust value < 0.05. Finally, we run Overrepresentation Analysis (ORA), Gene Set Enrichment Analysis (GSEA), and KEGG Pathways for protein-coding genes.
Results: We obtained 28.198 transcripts and 15.195 novel transcripts using the transcriptome-guided approach. We found 1.692 upregulated and 2.680 downregulated genes. Also, we identified 1.371 lncRNAs, and approximately 239 were differentially expressed between conditions. Additionally, we found hypertrophic markers genes upregulated, such as *Nppa*, *Nppb* and *Rcan1*, corroborating that the hypertrophic stimulus worked. Some of the predominant Biological Process terms in ORA were “muscle system process” and “heart process”; KEGG terms were enriched in “MAPK signaling”, “Adrenergic signaling in cardiomyocytes”, among others. Finally, GSEA showed some activated processes, such as “signaling receptor activity” and “mitochondrial processes,” that were mainly suppressed.
Conclusions: Due to poor annotation of isoforms and non-coding genes in rat model, these results improve the characterization of non-coding transcripts in rat genome using a reconstruction transcript pipeline coupled with several coding potential algorithms in a PCH context.
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163. Effect of the Cold Shock Protein CspA on the Folding Mechanisms of 5’UTR-CspA mRNA
Cristian Valdebenito (cristian.valdebenito3@gmail.com), Cayetana Zamorano, Rodrigo Rivera, & Mauricio Baez. Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.

Introduction: The CspA protein of *E. coli* functions as an RNA chaperone and its mRNA acts as a molecular thermometer to detect temperature changes. At low temperatures (10 °C), the 5’UTR mRNA forms a stable structure that enhances CspA translation. However, CspA induces a conformational change in the 5’UTR, resulting in a pseudoknot structure that inhibits its translation. The molecular mechanism underlying this feedback has not been fully explored.
Materials and Methods: To investigate how CspA triggers this conformational change, we used optical tweezers to mechanically unfold the 5’UTR RNA at 10 °C. This setup promotes the pseudoknot conformation of the 5’UTR mRNA, allowing us to observe the effect of CspA.
Results: Mechanical unfolding of the 5’UTR RNA revealed a single conformational transition between the extended and compact conformation. However, analysis of force-extension curves identified two alternative conformations, C1 and C2, with contour lengths of 38 nm and 62 nm, respectively. These lengths were shorter than the theoretical contour length (81 nm), indicating that the 5’UTR RNA adopts two partially unfolded structures. In the presence

of CspA, the population of C1 increased from 30% to 58%, while C2 decreased. Notably, this shift in population due to CspA did not affect the unfolding forces of C1.
Discussion: The increase in C1 population could be attributed to preferential binding of CspA. However, this is unlikely, as we did not observe an increase in the unfolding forces of C1. Therefore, our results suggest that CspA alters the folding route of the 5'UTR, favoring the C1 conformation. The implications for co-transcriptional regulation of this molecular thermometer are discussed.
Acknowledgment: FONDECYT 1231276.

164. Assessment of vascular remodeling and senescence in a mouse model heart failure with preserved ejection fraction. Francisca Valenzuela Arce[1](f.valenzuela.2@ug.uchile.cl), David Silva[1], Fernanda Sanhueza-Olivares[1], Andrea Mella-Torres[1], Angelica Ortega-Muñoz[1], Mayarling F. Troncoso[1, 2], Claudia Muñoz[1], Francisco Pino de la Fuente[1], Ximena Calle-Chalco[1, 3], Alejandra Hernández[1], Elsa Rocio Bascuñan[4], Javiera Martinez[1] , Sergio Lavandero[1, 5], Mario Chiong[1]. [1]University of Chile, Advanced Center for Chronic Diseases, Faculty of Chemical and Pharmaceutical Sciences, Santiago, Chile. [2]University of Chile, School of Medical Technology, Faculty of Medicine, Santiago, Chile. [3]University of O'Higgins, Institute of Health Sciences, Rancagua, Chile. [4]University of Andres Bello, Faculty of Life Sciences, Santiago, Chile. [5]University of Texas, Cardiology Division, Southwestern Medical Center, Dallas, USA.

Introduction: Heart Failure with preserved ejection fraction (HFpEF) is a syndrome characterized by diastolic dysfunction with a high prevalence in Chile. HFpEF is associated with comorbidities such as obesity and hypertension. Macro and microvascular dysfunction are normally found in HFpEF patients. However, vascular remodeling and its association with senescence are not described in HFpEF.
Objective: To evaluate vascular remodeling and senescence in aortic tissue obtained from a murine model of HFpEF.
Methods: Aortas were obtained from an HFpEF mouse model fed with a high-fat diet (HFD, 60% Fat) and Nω-nitro-l-arginine methyl ester (L-NAME, 1,5 g/L) for 15 weeks. The experimental groups were: control (chow diet), obese (HFD), and HFpEF (HFD + L-NAME). In aorta tissue sections, morphology, fibrosis, and senescence were assessed by hematoxylin-eosin, picrosirius red staining, and immunohistochemistry of P53, respectively.
Results: Aortic tissue of HFpEF mice presented a significant thickening of the arterial wall (control: 67.1± 5.7 µm, HFpEF: 75.4± 7.9 µm, $p<0.05$, n=22) and focal areas with vascular remodeling. Moreover, although collagen-positive regions were observed in aortic tissue in HFpEF mice, these areas were not significantly different from those of HFD and control mice. Finally, the aortic tissue presented an increase in p53 positive nuclei compared with their respective area (Control: 0,5±1,1 positive nucleus x 10^{-8}/total nucleus/ μm^2, 3.3± 3.9 positive nucleus x 10^{-7}/total nucleus)/μm^2. $p<0.05$, n=38).

Conclusion: In aortic tissue from HFpEF mice, there were increased levels of vascular remodeling and p53-positive cells compared to control mice.
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165. Bioenergetic changes induced by Endothelin-Converting Enzyme-1c in colorectal cancer cells. Javiera Vargas V.[1] (javiera.vargas.v@mail.pucv.cl), Paula Romero Vicencio[1], María de los Ángeles Toro[1], Karla Villalobos-Nova[1], Eduardo Silva-Pavez[2], & Julio C. Tapia[1]. [1]Laboratorio de Transformación Celular, Instituto de Ciencias Biomédicas (ICBM), Facultad de Medicina, Universidad de Chile. [2]Facultad de Odontología y Ciencias de la Rehabilitación, Universidad San Sebastián.

Introduction: The Endothelin-Converting Enzyme-1 isoform c (i.e., ECE-1c) is increased in many types of cancer. Mutation of a putative ubiquitinable Lys-6 to Arg (i.e., ECE-1c^{K6R}) at its cytosolic N-terminal end provokes the blockage of its proteasomal degradation. In addition, ECE-1c^{K6R} expression leads to the occurrence of cancer stem cells (CSCs) traits and augmented aggressiveness in different types of cancer cells, such as colorectal, lung, and glioblastoma. However, whether this cell transformation is related to an enhanced metabolic transformation of colorectal cancer cells is unknown. Since a trait of colorectal stem cancer cells is a preference for oxidative phosphorylation (OXPHOS), we aimed to investigate whether the ECE-1c^{K6R} expression is associated with mitochondrial changes and increase of OXPHOS rate in DLD-1 colorectal cancer cells.
Materials and Methods: Mock (empty vector), wild-type (ECE-1c^{WT}), and highly stable (ECE-1c^{K6R}) expressing clones were developed in DLD-1 cells. Proteins associated with mitochondrial biogenesis, relative mtDNA copy number, capacity to form colonies in adhesion, and mitochondrial mass were measured.
Results: Highly stable ECE-1c^{K6R} expression promoted increased mitochondrial biogenesis and activity in DLD-1 colorectal cancer cells. In addition, ECE-1c^{K6R} expression seemed to enhance metabolic adaptability when cells were exposed to grow in the absence of glucose.
Discussion: These results suggest that the occurrence of CSC traits in DLD-1 colorectal cancer cells is linked to an improved metabolic adaptability, with a preference for an oxidative phenotype.
Acknowledgment: FONDECYT grants #3220604 (ES-P) & #1220353 (JCT).

166. Cell Wall remodeling in Blueberry Fruits during ripening and in response to Methyl-Jasmonate (MeJA) treatments. Carlos Vásquez-Rojas[1,2] (carlos.vasquez6@cloud.uautonoma.cl), Ricardo I. Castro[3], Marcelo Muñoz-Vera[3], Luis Morales-Quintana[1]. [1]Multidisciplinary Agroindustry Research Laboratory, Instituto de Ciencias Biomédicas, Facultad de Ciencias de la Salud, Universidad Autónoma de Chile. [2]Programa de Doctorado en Ciencias Biomédicas, Instituto de Ciencias Biomédicas, Facultad de Ciencias de la Salud, Universidad Autónoma de Chile. [3]Multidisciplinary

Agroindustry Research Laboratory, Instituto de Ciencias Aplicadas, Facultad de Arquitectura, Construcción y Medio Ambiente, Universidad Autónoma de Chile.

Introduction: Fruit softening is a primary quality attribute in blueberries during ripening. This process is mainly a consequence of the solubilization and depolymerization of cell wall components mediated by a complex set of enzymes and proteins. Methyl jasmonate (MeJA) is a plant hormone known to play a crucial role in fruit defense against biotic and abiotic stress. This study presents a comparative analysis of physiological changes, polysaccharide content in the cell wall, and thermogravimetric analysis (TGA) during ripening stages as well as in MeJA-treated blueberry (*Vaccinium corymbosum* cv. 'O´Neil') fruits.
Materials and Methods: Fruits from *Vaccinium corymbosum* were harvested and categorized into three ripening stages. MeJA treatments were applied to some plants, while control plants received water. Physiological parameters, including water content, fruit size, soluble solids, and color, were measured. TGA and DSC analyses assessed fruit stability, and ATR-FTIR spectroscopy analyzed the chemical composition. Statistical analysis was conducted using ANOVA with $p < 0.05$ significance.
Results: Our results demonstrate a decrease in the stability values of cell wall polymers in MeJA-treated fruits, which correlated with the TGA curves. The TG analysis indicated that dried samples of treated fruits exhibit higher thermal stability, likely due to an increased number of hydrogen bonds between cell wall chains. Conversely, control fruits displayed lower thermal stability.
Discussion: These findings suggest a model for understanding cell wall changes in *V. corymbosum* during defense against various biotic and abiotic stresses. This study provides insights into the potential biochemical pathways involved in fruit ripening and defense, potentially aiding in the development of strategies to improve fruit quality and shelf life.
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167. Electrophysiological characterization of BDNF/TrkB signaling in hiPSC-CM and 3D bioengineered tissues. Leslye Venegas-Zamora[1,2], Abigail Giese[3], Matthew Fiedler[3,6], William Perez[3], Francisco Altamirano[3,4], Valentina Parra[1,2,5]. [1]Laboratory for Cell Differentiation and Metabolism, Department of Biochemistry and Molecular Biology, Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile. [2]Advanced Center of Chronic Diseases (ACCDiS), Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile. [3]Department of Cardiovascular Sciences, Houston Methodist Research Institute, Houston, Texas, USA. [4]Department of Cardiothoracic Surgery, Weill Cornell Medical College, Cornell University, Ithaca, NY, USA. [5]Systems Biology Center for the Study of Extremophile Communities from Mining Tailings (SYSTEMIX), O'Higgins University, Rancagua, Chile. [6]Program in Physiology, Biophysics and System Biology, Weill Cornell Graduate School of Medical Sciences, NY, USA.

Introduction: Cardiovascular diseases are increasing globally, with ischemic heart disease being the leading cause of death. Brain-derived neurotrophic factor (BDNF) and its receptor Tropomyosin-related kinase receptor B (TrkB) have emerged as cardioprotective factors that ameliorate cardiac damage and improve contractility in mouse models. We aimed to evaluate whether BDNF/TrkB signaling regulates electrophysiology and calcium (Ca^{2+}) signaling in human cardiomyocytes derived from iPSCs using bi- and tri-dimensional culture approaches.
Materials and Methods: iPSCs were differentiated into cardiomyocytes using dual Wnt modulation protocols, followed by maturation with thyroid hormone and dexamethasone. Excitation-contraction coupling was evaluated by assessing action potentials (FluoVolt), Ca^{2+} transients (Cal590), and contractility (contrast pixel correlation algorithm). Wave propagation in monolayers was assessed using Ca^{2+} optical mapping to evaluate the effects of BDNF on conduction. Engineered heart tissues (EHT) composed of iPSC-CM and human fibroblasts were used to determine BDNF-derived effects on cardiac contraction.
Results: T3/dexamethasone increased CM maturation, correlating with elevated TrkB mRNA levels. BDNF stimulation increased Ca^{2+} spark frequency and spontaneous Ca^{2+} activity in hiPSC-CMs. Interestingly, BDNF treatment increased spiral Ca^{2+} waves, characteristic of arrhythmic activity, likely due to RyR Ca^{2+} leaking. TrkB knockdown blunted these effects. In single cells, BDNF treatment did not alter calcium levels, action potential duration, or contraction. Conversely, in EHTs, BDNF treatment increased contraction, consistent with previous murine model findings, demonstrating the advantages of bioengineered tissues in modulating *in vivo* cardiac behavior.
Discussion: We used iPSC-CMs and EHTs to approximate human physiology. BDNF/TrkB signaling altered electrophysiological properties in 2D monolayers but not in single-cell measurements. Furthermore, BDNF increased contraction in 3D-cultured cardiomyocytes, suggesting that structural components may play a role. These findings enhance our understanding of the role of this trophic factor and its receptor in human cardiac physiology.
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168. Discovery and characterization of compounds that disrupt MYC/NSD3S oncogenic interaction. Sebastián Vera[1], Matías Hepp[1], Yuhong Du[2], Haian Fu[2] and Valentina González-Pecchi[1]. [1]Laboratorio de Investigación en Ciencias Biomédicas, Universidad Católica de la Santísima Concepción, Concepción, Chile. [2] Emory Chemical Biology Discovery Center, Emory University, Atlanta, USA.

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 more than 30 years. However, still there is no MYC inhibitor approved by the FDA. Recently, we described a new oncogenic interaction between MYC and NSD3S. In this project, our aim was to identify and characterized compounds that were able to disrupt MYC/NSD3S oncogenic interaction.
Material & Methods: We used ultra-high throughput screening (uHTS) assays, 1536 well plates expressing the target proteins to test different compounds from the MedChemExpress (MCE) library. The NanoLuc-based Protein-Fragment complementation assay (NanoPCA) was used to evaluate the interaction between MYC/NSD3S with or without compounds. Immunoprecipitation assays were used as a secondary assay to validate the positive hits found in the primary screening assay.
Results: We screened over 1000 compounds to identify inhibitors capable of disrupting the interaction between MYC/NSD3S. The percentage hit rate of the screening assay was 0,1%, with 12 compounds capable of modulating MY/NSD3S interaction, as well as decreasing MYC transcriptional activity as a secondary assay. The validation of the positive compounds was performed by immunoprecipitation, locking at MYC/NSD3S interaction.
Discussion: Our discoveries highlight a list of promising compounds for disrupting MYC/NSD3S interaction and introduce a novel approach for identifying and validating inhibitors of protein-protein interactions. By integrating NanoPCA with immunoprecipitation, 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.
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169. Thermal stabilization of R-Phycoerythrin by cross-linking with polyphenols.
Vera-Oñate Valentina (vvera1016@udec.cl), Alarcón-Fica Isabel, Castro-Caris Felipe, Martínez-Oyanedel José. Laboratorio de Biofísica Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.

The red algae *Agarophyton chilensis* (*Pelillo*) captures and absorbs light energy through the phycobilisome (PBS), a protein complex built of phycobiliproteins (PBP's) and linker proteins. Phycoerythrin (PE), phycocyanin (PC), allophycocyanin (PC) are colored proteins by the covalent binding of linear tetrapyrroles that give it spectroscopic and fluorescent properties.
Due to the characteristic coloration of PBP's, there is interest in the food industry in a possible replacement of synthetic dyes with dyes of natural origin.
The application of PE in food products is limited to preparations that involve low temperatures, because it has a first melting temperature (Tm) of 53 °C and a second at 72 °C, however, it has been described that cross-linking treatments with cross-linking agents improve its thermal stability, achieving higher Tms. For this work, *Pelillo* was collected on the shore of Coliumo beach, being washed and crushed in Ultra Turrax(™) T25 from IKA, obtaining a colored extract containing PBP's. To purify the proteins, the crude extract was

precipitated with 60% ammonium sulfate, then strong anion exchange and size exclusion chromatography, achieving PE with a purity greater than 3.5. Cross-linking was performed with polyphenols: Gallic acid, Epigallocathechin gallate, Pyrogallol, Tannic acid, Chlorogenic acid and Methyl glyoxal, the best stabilization result in an increase of around 10 °C after treatment.
The obtaining of dyes of natural origin seeks to replace the use of dyes of artificial origin because the latter can generate side effects such as hyperactivity in children, cancer and other conditions, if consumed for a long time. Furthermore, the use of PBPs as natural colorants promotes the generation of additional value to algae, which today is marketed only as raw material for obtaining hydrocolloids.
Acknowledgment: VIU23P0113.

170. Targeting Endothelin-1 metabolism to reduces tumor progression markers in gallbladder cancer cells. Jetzabel Vidal-Vidal, David Brown-Brown, Valentina Ojeda, Carlos Spichiger, Gaspar Peña, Ignacio Niechi. Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile.

Introduction: Gallbladder cancer (GBC) ranks among the most prevalent and lethal cancers within the biliary tract in our country. Early-stage GBC is often asymptomatic, leading to high therapeutic failure rates with conventional treatments. Therefore, identifying new therapeutic targets to mitigate malignant progression is imperative. One significant pathway implicated in tumor progression is the Endothelin-1 (ET1) pathway, which signals through G protein-coupled receptors (ETRs) to promote tumor growth. ET1 is produced from Big-ET1 by Endothelin-converting enzyme 1 (ECE1) and is irreversibly degraded by Neprilysin (NEP). Consequently, employing recombinant NEP (rNEP) and inhibiting ECE1 could reduce ET1 levels, thereby diminishing markers and genes associated with malignancy.
Materials and Methods: we utilized the NOZ cell line and primary CAVE1 culture. Epifluorescence microscopy was employed to assess calcium levels post-ET1 stimulation, indicating ET1 signaling activation. Additionally, treatments with rNEP and the ECE1 inhibitor SM19712 were conducted, followed by evaluation of tumor progression markers by western blot and RT-qPCR.
Results: rNEP application and ECE1 inhibition reduced ET1 pathway activation markers and the expression of target genes linked to tumor progression. Furthermore, exogenous ET1 triggered calcium release, confirming receptor-mediated signaling.
Conclusion: Targeting the production and degradation of ET1 significantly reduces markers and genes associated with tumor progression in gallbladder cancer.
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171. Proapoptotic effect of erioflorin and erioflorin acetate in castration-resistant prostate cancer. Cecilia Villegas[1] (c.villegas04@ufromail.cl), Iván González-Chavarría[2]

and Cristian Paz[1]. [1]Laboratory of Natural Products & Drug Discovery, Department of Basic Sciences, Universidad de La Frontera, Temuco 4780000, Chile. [2] Laboratory of Lipoproteins and Cancer, Pathophysiology Department, Universidad de Concepción, Concepción 4030000, Chile.

Introduction: Castration-resistant prostate cancer (CRPC) represents a significant challenge in oncology. Despite advances in treatment, resistance to hormonal therapies limits therapeutic options. In this context, the search for new compounds with antitumor activity is crucial. Germacran sesquiterpene lactones (GSLs) have shown promising results in various types of cancer. In this study, we evaluated the antitumor potential of Erioflorin and Erioflorin acetate, GSLs isolated from *Podanthus mitiqui*, on CRPC cell lines.
Materials and Methods: CPRC cell lines 22Rv1 and DU-145 were used. To assess cytotoxicity, a real-time cell death assay was performed using IncuCyte. Induction of apoptosis was determined by measurement of ROS, mitochondrial membrane potential and quantification of apoptotic cells by flow cytometry. Expression of pro- and anti-apoptotic genes (*BAX* and *BCL-2*) was analyzed by RT-qPCR.
Results: Erioflorin and Erioflorin acetate showed potent cytotoxic activity at 48 h of treatment. Erioflorin acetate was the most potent in both cell lines (IC_{50}: 27.3 and 35.1 µM), while Erioflorin showed lower activity (IC_{50}: 50.3 and 56.5 µM). In addition, both compounds induced a significant increase in ROS generation during the first 5 hours and a decrease in mitochondrial membrane potential, events characteristic of apoptosis. Flow cytometry analysis at 20 h of treatment confirmed an increase in the percentage of cells in early and late apoptosis. Finally, increased expression of *BAX* was observed, suggesting activation of the intrinsic apoptosis pathway.
Discussion: These results demonstrate that Erioflorin and Erioflorin acetate exhibit a potent proapoptotic effect in CRPC cells. These compounds induce cell death through mechanisms involving ROS generation and mitochondrial disruption. These GSLs could be considered promising candidate molecules for the development of new therapies against CRPC. However, *in vivo* studies are needed to confirm and elucidate in detail the underlying molecular mechanisms.
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172. Phenotypic Plasticity in Cistanthe longiscapa: A Pioneering Model for Drought Tolerance in Cultivars. Ricardo Yusta[1,2] (ryustac@gmail.com), Sebastián Zuñiga[1], Jorge Caballero[1], Gabriela Vásquez[1], Alejandro Moya[1], Juan Pablo Parra[1], Álvaro Miquel[1], Ariel Orellana[1,2]. [1]Centro de Biotecnología Vegetal, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago, Chile. [2]Instituto Milenio Centro de Regulación del Genoma (CRG), Santiago, Chile.

Climate change poses significant challenges for agriculture, with drought being a major threat. Enhancing water use efficiency (WUE) in crops is essential, as CAM (Crassulacean Acid Metabolism) plants are about 40% more efficient in WUE than C3 plants. Since most agronomic crops are C3, improving their WUE is critical. Understanding how CAM plants adapt to extreme conditions can inform strategies to develop drought-tolerant varieties. Cistanthe longiscapa, an iconic annual plant of the Atacama Desert's blooming phenomenon, thrives for up to three months under extreme conditions.
Our previous studies revealed that C. longiscapa can perform varying intensities of CAM photosynthesis based on local precipitation levels, demonstrating phenotypic plasticity. We further evaluated this plant's drought tolerance in laboratory conditions, exposing it to drought for up to six weeks and assessing its photosynthetic performance. Remarkably, plants under drought maintained active photosystem II but exhibited almost closed stomata after 11 hours of light exposure and again after 11 hours in darkness, indicating extreme CAM behavior. Conversely, well-irrigated plants showed active photosystem II similar to drought-exposed plants but had high stomatal aperture after 11 hours of light and nearly closed stomata after 11 hours of darkness, resembling C3 plants.
These findings highlight C. longiscapa's ability to switch from C3 to extreme CAM photosynthesis based on water availability, showcasing its phenotypic plasticity. This adaptability makes C. longiscapa an excellent model for studying molecular mechanisms to enhance WUE in crops, providing insights that could lead to the development of drought-resistant varieties.
Acknowledgment: ANID – Millennium Science Initiative Program – ICN2021_044; Mizutani Foundation for Glycoscience.

173. Overexpression of FchNAC1 induces color and the phenylpropanoid/anthocyanin pathway. Macarena Zamorano-Curaqueo[1,2] (mzamorano@utalca.cl), Raúl Herrera[1], María Alejandra Moya-León[1]. [1]Functional Genomics, Biochemistry and Plant Physiology Group, Instituto de Ciencias Biológicas, Universidad de Talca. [2]Doctorado en Ciencias, mención Ingeniería Genética Vegetal, Universidad de Talca.

Introduction: The NAC transcription factor (TF) family is unique to plants and regulates various processes, including plant growth, development, stress tolerance and fruit ripening. FchNAC1 was described in *Fragaria chiloensis* fruits (Carrasco-Orellana et al., 2019) showing to transactivate the *FchPL* promoter, a gene involved in cell wall remodeling. This suggests that FchNAC1 could play crucial roles in development and ripening of Chilean strawberries. The aim of this work was to evaluate the effect of FchNAC1 overexpression on the development of color, an important quality attribute of strawberry fruit, and therefore transcriptional changes in the phenylpropanoid/anthocyanin pathway were quantified.
Materials and Methods: *Fragaria chiloensis* fruits under development (stage 2) were agro-infiltrated with an overexpression vector containing the *FchNAC1* sequence or the empty

vector (EV). Fruit tissues were collected after two days, frozen and stored at -80°C. Total RNA was extracted from fruit tissues and cDNA was synthesized. The level of transcripts codying for genes of the phenylpropanoid/anthocyanin pathway were quantified by RT-qPCR.
Results: *FchNAC1* overexpressing fruits display a rise in red color (color a) compared to EV fruit. Gene expression analysis revealed in *FchNAC1* overexpressing fruits an increment in transcript levels of key genes of the phenylpropanoid pathway, such as *FchPAL2*, *Fch4CL* and *FchCHS*, and in those driving towards the biosynthesis of anthocyanins such as *FchANS* and *FchUFGT*, compared to EV fruit. Interestingly, there was also a rise in the expression of genes leading to proanthocyanidin synthesis, *FchANR* and *FchLAR*.
Discussion: These results demonstrate that FchNAC1 can regulate the expression of a series of genes of the phenylpropanoid/anthocyanin pathways leading to the synthesis of anthocyanins, important secondary metabolites with antioxidant properties related to color and health.
Acknowledgment: FONDECYT 1210948 and ANILLO ACT210025 grants.

174. Study of ADP-ribosylation of *de novo* synthesized histones H4. Patricio Zapata (pzapatac1@correo.uss.cl)[1], Yerko Castillo[1], Rodrigo Villanueva[1], Ignasi Forme[2], Axel Imhof [3], & Alejandra Loyola[1]. [1]Department of Basic Sciences, Faculty of Medicine and Sciences, Universidad San Sebastián, Santiago de Chile, Laboratory of Epigenetics and Chromatin, Fundación Ciencia & Vida. [2]Protein Analysis Unit, Biomedical Center, Faculty of Medicine, Ludwig-Maximilians-University (LMU) Munich, Martinsried 82152, Germany. [3]Biomedical Center Munich, Department of Molecular Biology, Faculty of Medicine, Ludwig-Maximilians Universität München, Planegg-Martinsried, Germany.

Introduction: Histones ADP ribosylation (ADPr) involves the covalent transfer of polyADPr (PAR), catalyzed by PARP1. This modification is crucial for DNA damage repair. In the cytoplasm, ADPr occurs on newly synthesized histones H3 and H4 before they associate to form the dimer. By binding into an H3-H4 dimer via the "histone fold", they lose ADPr. However, it is not known the function of this modification. This study investigates the initiation of PAR formation on newly synthesized histone H4, as well as the role of this mark, and the enzymes involved in this process.
Materials and methods: We performed mass spectrometry analysis of post-translational modifications of the histone H4 associated to the ribosome of HeLa cells. This was followed by an assay using UNC0379, a selective inhibitor of SETD8, to obtain cellular extracts and perform ribosomal profiling, which was analyzed by western blot. To determine whether H4 PTMs found at ribosomes could recruit PARP1, we perfomr PARP1 pull-down with H4 peptides, H4k20me1 and H4K20me3. The assays were analyzed by Western blot.
Results: K20me1 of nascent H4 was identified by MS analysis. RIPA assays showed an increase in PARP1 levels and a decrease in H4 and H4K20me1 in treated cells compared to control cells. In the sucrose gradient, PARP1 was detected together with ribosomal

proteins, and H4 was found in different fractions in treated cells versus control cells. Pull-down assays identified the presence of PARP1 associated with the H4K20me1 peptide.
Discussion: Nascent H4 was identified to be present in K20me1, PARP1 and ribosomal proteins in the fractions derived from the sucrose gradient. Pull-down assays showed a higher affinity of PARP1 for monomethylated histones.
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175. The effect of selenium deficiency on HFD-induced cardiac ferroptosis. Úrsula Zúñiga-Cuevas[1,2] (ursula.zuniga@ug.uchile.cl), Xuanxuan Guo[3], Nils Bömer[3], Valentina Parra[1,2,4]. [1]Laboratorio de Diferenciación Celular y Metabolismo, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile. [2]Advanced Center for Chronic Diseases (ACCDiS), Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile. [3]Department of Cardiology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands. [4]Systems Biology Center for the Study of Extremophile Communities from Mining Tailings (SYSTEMIX), O'Higgins University, Rancagua, Chile.

Introduction: Selenium deficiency (SD) is associated with several health issues, including increased susceptibility to oxidative stress and inflammation. In obesity, SD may exacerbate lipid peroxidation and tissue damage, promoting ferroptosis, a distinct type of cell death. These molecular events triggered by SD are risk factors for cardiovascular diseases. SD has been linked to cardiomyopathy, while high serum selenium levels correlate with lower mortality in heart failure patients. However, the mechanisms of SD-induced ferroptosis remain unclear. This work aims to determine the effect of SD on obesity-associated cardiac ferroptosis.
Materials and Methods: Adult C57BL male mice were kept under a normal diet (LFD) or high-fat diet (HFD) with either deficient or sufficient levels of selenium supplementation for eleven weeks. After this, the animals were euthanized, and the left ventricle was isolated to evaluate mRNA levels of 17 selenoproteins along with ferroptosis markers. In addition, neonatal rat cardiomyocytes were treated with palmitate ir order to emulate an *in vitro* model of HFD, and different markers were also assessed to characterize ferroptosis.
Results: SD is associated with lower mRNA levels of selenoproteins Glutathione peroxidase 1 (GPX1), Thioredoxin reductase 2 (TR2), and Selenoprotein H (Sel H) compared to sufficient selenium levels in LFD conditions and a higher expression of the ferroptosis marker Transferrin receptor 1 (TFR1) in the HFD conditions.
Discussion: These results highlight the possibility that SD can promote or worsen a ferroptotic state
under HFD conditions in the heart due to a lower expression of selenoproteins regulating oxidative stress. Further investigation is required to determine which selenoproteins crucially

regulate ferroptosis in cardiac cells and how the modulation of selenium levels could prevent the progression of cardiovascular diseases.

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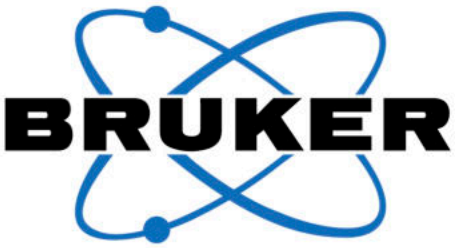

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