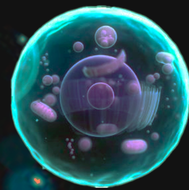
XLVI Annual Meeting

Chilean Society for Biochemistry and Molecular Biology



2023

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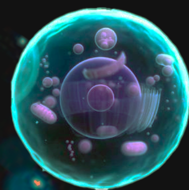

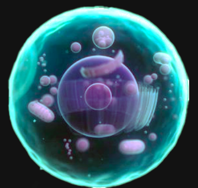

2023

3rd - 6 th October
Hotel Club, La Serena
Región de Coquimbo

XLVI Annual Meeting Chilean Society for Biochemistry and Molecular Biology

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ABSTRACTS

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OPENING LECTURE

Of Mice and Men: What can genetically engineered mouse models teach us about human cancer?

Daniel Murphy. School of Cancer Sciences, University of Glasgow, Scotland. CRUK Beatson Institute for Cancer Research.

Mouse models of cancer are a mainstay of modern cancer research but their ability to accurately reflect human cancer biology varies across a broad spectrum. From simple xenografts to the most sophisticated genetically engineered mouse models (GEMMs), key aspects of human cancer biology are typically absent or poorly reflected. With a focus on thoracic cancers, namely Lung Adenocarcinoma and Mesothelioma, the Murphy lab has developed new GEMMs to begin to address some of the shortcomings of heretofore available GEM models, and through comparative molecular phenotyping to align specific models with subsets of corresponding human cancers. Our model systems are by definition works in progress but begin to shed light on new opportunities for early detection of cancer and to serve as disease- relevant platforms for preclinical testing of therapeutic combinations stratified for specific molecular subtypes of thoracic cancers. The lecture will additionally emphasise the causative link between asbestos exposure and Mesothelioma, raising awareness of a preventable cancer of growing concern in India and elsewhere.

OSVALDO CORI LECTURE

A good cocktail: kinases, metabolism, and evolution in proteins from extremophiles.

Victoria Guixé, Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

In extreme environments, a particular biota named Archaea is predominant. Life in these harsh conditions imposes many challenges, mostly concerned with protein structure and enzyme activity. One interesting question that will be addressed is how the proteins and enzymes of such organisms have evolved to cope with these extreme conditions, identifying the structural and functional traits that allow protein adaptation through evolution in the ADP-dependent kinase family from archaea. Also, since in this protein family there are specific and bifunctional enzymes, we addressed changes in substrate specificity during evolution along with the structural determinants of these changes. This allows us to explore the mechanism underlying the change in protein function during natural evolution, illuminating the evolutionary basis for substrate recognition. Moreover, archaeal organisms present a metabolic complexity that includes the presence of unique pathways, like methanogenesis, and some modified-pathway versions of the classical routes, such as the Embden-Meyerhof-Parnas (glycolysis) which is highly regulated in all the organisms studied. Although it has been claimed that enzyme regulation in Archaea occurs mainly at the transcriptional level, we will present evidence regarding allosteric regulation of a bifunctional PFK/GK ADP-dependent kinase from a methanogenic organism, which includes determination of the kinetic activation mechanism, the evolutionary trajectory of this trait,

and its structural basis. The results suggest that although AMP activation is conserved along the evolution of this protein family, the underlying mechanism is not conserved.

SEVERO OCHOA LECTURE

Targeting connexins to overcome drug resistance in cancer. María D. Mayán. Instituto de Investigación Biomédica A Coruña (INIBIC). A Coruña, España

Drug resistance is a major challenge in modern cancer therapy, despite the significant advances made with targeted and immunotherapies for various cancers, including advanced BRCA1/2 or BRAF mutation-positive tumors. However, these treatments often have limited efficacy and are vulnerable to drug resistance. Our group has identified a new target that plays a critical role in cell-to-cell communication and enhances the effectiveness of targeted therapies involving DNA damage, such as PARP inhibitors. By recruiting DNA repair complexes to lamina-associated domains and promoting persistent DNA damage, this target contributes to genome instability and synthetic lethality resulting from excessive DNA damage. Our research suggests a novel therapeutic approach for overcoming drug resistance in these tumors. We have designed an innovative drug combination that uses extracellular vesicles to deliver the mRNA of the identified target, exploiting this vulnerability to enhance cell death and the anti-tumor immunity in combination with targeted therapies and immunotherapies. Our findings highlight a new player in DNA repair and drug response in the tumor context, with significant potential to improve treatment outcomes for patients with advanced tumors by overcoming the limitations of current therapies.

SYMPOSIUMS

DIFFERENT ANGLES TO GASTRIC CANCER AND RELATED PREVALENT DISEASES SYMPOSIUM.

Gastro-esophageal cancers at the interface of biology and new therapeutic options. Wael El-Rifai, MD, PhD (wxe45@miami.edu). Sylvester Comprehensive Cancer Center, University of Miami Miller School of Medicine, Miami, FL, USA.

On behave of the Gastro-esophageal Cancer Team:
Marwah Al-Mathkour, PhD, Farah Ballout, PhD, Lei Chen, PhD, Melanie Genoula, PhD, Ahmed, Gomma, MD, PhD, Tianling Hu, Heng Lu, PhD, Selma Maacha, PhD, Dunfa Peng, PhD, Nadeem Sidiq Bhat, MSc, Mohammed Soutto, PhD, Shoumin Zhu, PhD, Longlong Cao, MD, PhD, Oliver G. McDonald, MD, Alexander Zaika, PhD, Xi Steven Chen, PhD

Gastro-esophageal adenocarcinoma (GEACs) are aggressive malignancies highly resistant to current therapeutic approaches. Although these cancers are characterized by remarkable genomic alterations and chromosomal instability, targeted therapy directed towards commonly amplified or mutated genes has not met the expectations to improve clinical outcomes. Receptor tyrosine kinase (RTK) signaling is commonly activated in GEACs. We discovered that RTK activation is associated with mesenchymal-type gastric cancers (GC). We developed a bioinformatics algorithm and re-stratified subtypes of GC in public databases. Immune desert- and excluded-type tumors are resistant to immune checkpoint inhibitors (ICIs) compared with immune-inflamed GCs. Moreover, epithelial-mesenchymal transition (EMT) signaling was highly enriched in immune desert-type GC. Syngeneic murine tumors exhibiting mesenchymal-like properties, compared with epithelial-like, are T cell-excluded and resistant to CTLA4 blockade. Dovitinib, and RTK inhibitor sensitized the desert-type immune-cold GC to CTLA4 blockade by restricting EMT, modulating the tumor microenvironment (TME), and recruiting T cells. Our work in EAC has shown that mesenchymal type EAC is enriched for redox signaling, showing activation of redox factor 1 (REF1). The mesenchymal phenotype of EAC was dependent on the redox activity of REF1, where its inhibition was effective in decreasing the tumor burden in EAC patent-derived xenografts. The relationship between redox and RTK signaling, as they relate to immune modulation in the TME in EAC, is undergoing additional investigation using pre-clinical models, paving the way to clinical trials in patients.
Conclusion: Molecular subtyping and reclassification of GEACs identifies druggable targets and pathways that can be applied to improve the clinical outcome in these patients

Funding:
National Cancer Institute: R01CA131225, R01CA133738, R01CA206563, R01CA249949, and P01CA268991)

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Competing endogenous RNA networks in gastric cancer. Wilda Olivares[1], Pablo Santoro[1], Francisco Carvajal[1], Arnoldo Riquelme[2], Andrew F.G. Quest[3,4], Alejandro Corvalan[1] (acorvalan@uc.cl). [1]Advanced Center for Chronic Diseases (ACCDiS) and Department of Hematology and Oncology, Pontificia Universidad Catolica de Chile. [2]Department of Gastroenterology and Center for Prevention and Cancer Control (CECAN), Pontificia Universidad Catolica de Chile. [3]Advanced Center for Chronic Diseases (ACCDiS), Universidad de Chile. [4]Laboratory of Cell Communication, Center for studies on Exercise Metabolism and Cancer (CEMC), Faculty of Medicine, Universidad de Chile

The unexpected decline in gastric cancer (GC) mortality was abruptly reversed with the arrival of SARS-CoV-2 in 2020. A large surge in diagnosed new cases has already begun to result in worse cancer stages and survival outcomes, a new reality, which underscores the urgency of implementing novel science-based interventions. The premalignancy of GC, a stepwise progression of well-defined histological lesions, after Helicobacter *pylori* (H.*pylori*) infection offers a unique opportunity for interception. Through an in silico "hypothesis generating research pipeline", we discovered and began the characterization of the novel long non-coding gene KCNQ1 opposite strand 1 (KCNQ1OT1) in GC. KCNQ1OT1 is the largest monoexonic gene in the human genome, spanning 91,671 nucleotides, with a potential dual role in GC through a competing endogenous RNA (ceRNA) network in the cytoplasm and DNA hypermethylation in the cell nucleus. We found that KCNQ1OT1 is overexpressed in GC and associated with worse overall survival upon upregulation of invasiveness genes by ceRNA networks. We posit that KCNQ1OT1 may be related to cellular senescence in the premalignancy of GC, since preliminary data show co-expression of KCNQ1OT1 and upon H. pylori infection, *in vitro*. In addition, ceRNA networks involving KCNQ1OT1 and senescence transcripts were found, *in silico*. We also found KCNQ1OT1 in the nucleus, where it correlates with higher DNA methylation levels of senescence transcripts. We are exploring if this dual role of KCNQ1OT1 drives the stepwise progression from senescence to neoplasia in the premalignancy of GC.

Funding: Fondecyt 1231773 and CONICYT FONDAP 15130011

Local gastric and systemic effects of *Helicobacter pylori*. Andrew F.G. Quest[1,2] (aquest@u.uchile.cl), Manuel Valenzuela[2,3], Denisse Bravo[2,4] and Lisette Leyton[1,2]. [1]Laboratory of Cellular Communication, Center for Studies On Exercise Metabolism and Cancer (CEMC), Institute of Biomedical Sciences (ICBM), Facultad de Medicina, Universidad de Chile, Santiago, Chile. [2] Advanced Center for Chronic Diseases (ACCDiS), Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile. [3] Laboratorio de Microbiología Celular, Instituto de Investigación y Postgrado, Facultad de Ciencias de La Salud, Universidad Central de Chile, Santiago, Chile. [4] Cellular Interactions Laboratory, Faculty of Dentistry, Universidad Andres Bello, Santiago, Chile

Helicobacter pylori (H. pylori) infection affects roughly 50% of the world population and approximately 70% of the population in Chile. While infection with this bacterium is considered the major risk factor associated with the development of intestinal type gastric cancer, it is also known to trigger systemic effects. Here, I will elaborate on both aspects of *H. pylori* infection. Effects of *H. pylori* infection are attributed to the presence of virulence factors. Here, I will first discuss our findings related to how *H. pylori* infection promotes gastric cell death due to loss of the protein Survivin, via a mechanism involving the gamma glutamyl transpeptidase virulence factor. I will also present evidence indicating that *H. pylori* triggers the release of extracellular vesicles (EVs) from infected cells that can alter the behavior of neighboring non-infected cells. Then, I will discuss how *H. pylori* also triggers a survival response in infected cells that leads to induction of the hypoxia induced factor-1α (HIF-1α) via a mechanism involving the Urease virulence factor, an enzyme produced by the bacteria to neutralize the acidic stomach environment and permit survival. Finally, I will discuss our observations showing that *H. pylori* also produces vesicles called Outer Membrane Vesicles (OMVs), highly enriched in Urease, that can escape from the stomach environment and reach the brain where they induce astrocyte reactivity and neuronal damage. Thus, our observations shed light on mechanisms by which this highly prevalent human pathogen not only triggers local gastric but also induces systemic effects.

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HIF1α/Rab5/β-catenin: a novel signaling axis triggered by Helicobacter pylori in gastric cancer cells. Daniela Herrera[1,2,3,4], Héctor Tapia[2,3,4,5], Denisse Bravo[2,5], Andrew F. G. Quest[1,2], Vicente A. Torres[2,3,4] (vicentetorres@uchile.cl). [1]Laboratory of Cellular Communication, Center for studies on Exercise, Metabolism and Cancer (CEMC), Faculty of Medicine, Universidad de Chile, Santiago, Chile; [2]Advanced Center for Chronic Diseases (ACCDiS), Santiago, Chile; [3]Institute for Research in Dental Sciences, Faculty of Dentistry, Universidad de Chile, Santiago, Chile; [4]Millenium Institute on Immunology and Immunotherapy (MIII), Santiago, Chile; [5]Universidad Andrés Bello, Santiago, Chile.

Helicobacter pylori infection has been associated with the etiology of intestinal-type gastric cancer via different virulence factors. Previously, a non-enzymatic role of urease was described, which promoted stabilization of the hypoxia inducible factor, HIF-1α, a key protein associated with tumor progression and metastasis. More recent studies by our group showed that HIF-1α transcriptionally induces the Rab-guanine exchange factor ALS2, activating the small GTPase Rab5 and hence increasing tumor cell migration, invasion, and metastasis. Alternatively, HIF-1α is known to interact with β-catenin, thereby promoting expression of a subset of genes required for tumor cell invasion. In my presentation, I will show results that explored the relevance of HIF-1α in Helicobacter pylori-triggered events, specifically the activation of Rab5 and β-catenin-dependent signaling. Our data indicate that Helicobacter pylori increases ALS2 and Rab5-GTP levels in gastric cancer cells and that these events depend on HIF-1α stabilization. Interestingly, Rab5 activation is associated with fluctuations in early endosome dynamics and sequestration of signaling protein regulators, such as GSK3β. Endosomal localization of GSK3β -a component of the β-catenin destruction complex- is followed by nuclear localization of β-catenin in gastric cancer cells infected with Helicobacter pylori. Intriguingly, most of HIF-1α stabilized upon infection by Helicobacter pylori is detected in the nuclear compartment and found to associate with β-catenin in a protein complex. To what extent these events associated with changes at the transcriptional level are relevant to gastric cancer development and progression remains to be defined.

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BIOCHEMICAL AND MOLECULAR MECHANISMS INVOLVED IN THE INTERACTION OF BENEFICIAL MICROORGANISMS WITH PLANTS IN THE CLIMATE CHANGE CONTEXT.

Regulation of the bacterial community and production of acyl homoserine lactones and their relationship with Antarctic vascular plants under a climate change scenario.

Claudia Rabert[1] (claudiarabert@gmail.com), Alejandra Fuentes-Quiroz[2], Daisy Tapia-Valdevenito[1], Giovanni Larama[3]. [1]Instituto de Ciencias Biomédicas, Universidad Autónoma de Chile, Temuco, Chile. [2]Laboratorio de Biorremediación, Facultad de Ciencias Agropecuarias, Universidad de La Frontera. [3] Laboratorio de Investigación en Biocontrol, Universidad de La Frontera, Temuco, Chile.

Deschampsia antarctica and *Colobanthus quitensis* are the only two species of vascular plants that naturally inhabit the Antarctic Peninsula. This inhospitable environment restricts their growth and reproductive period solely to the summer season. Given these conditions, the research objective is focused on exploring not only the particularities of these plant species but also the close relationship and interaction established successfully between soil, microorganisms, and plants. To achieve this, a defined transect of approximately 2.5 km in length was established, covering three sampling points from the coastal area of Admiralty Bay to the retreat of the Ecology Glacier. This allowed us to observe different soil and microclimatic conditions in the area. Analysis of macro and micronutrients was carried out, with corresponding records of temperature, humidity, and soil conductivity, along with the collection of rhizospheric soil to explore the microorganisms associated with the plant species through the analysis of 16S regions, as well as leaf material to highlight absorbed nutrients. To demonstrate the adaptations of the established soil-microorganism-plant interaction during the growing season, samples were taken both at the beginning (December) and the end (March) of this period. Additionally, the study delved into the detection of acyl-homoserine lactone (AHL) molecules, which are associated with bacterial quorum-sensing activity. Interesting results were obtained related to microclimatic and soil nutritional conditions, showing differentiating nutrients between sites and collection times, such as phosphorus, potassium, calcium, aluminum, and sodium. At the leaf level, aluminum, sodium, magnesium, calcium, and potassium exhibited distinctive responses. At the level of bacterial phyla and taxa, it was observed that the most abundant phyla generally remained consistent across sites. However, their proportions were affected in terms of differences between sites and collection times. Changes in diversity were also observed at the taxa level, which could be associated with the level of adaptation of the microorganisms present at each site. Additionally, AHL-type molecules were identified in bacterial isolates, which have been linked to promoting plant growth.

Auxin dynamics in *Serendipita indica* infected Arabidopsis roots. Adrián González Ortega-Villaizán[1]; Ralf Oelmüller[2]; Jesús Vicente Carbajosa[1,3]; Stephan Pollmann[1,3]. [1]Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid (UPM)–Instituto Nacional de Investigación y Tecnología Agraria y Alimentación (INIA/CSIC), Campus de Montegancedo, 28223 Pozuelo de Alarcón (Madrid), Spain. [2]Matthias Schleiden Institute of Genetics, Bioinformatics and Molecular Botany, Department of Plant Physiology, Friedrich-Schiller-University Jena, 07743 Jena, Germany. [3]Departamento de Biotecnología-Biología Vegetal, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid (UPM), 28040 Madrid, Spain.

Plants share their habitat with a wide variety of microbes. This close proximity has promoted the evolution of inter-organismic interactions between plants and microorganisms, providing mutual growth benefits to both the plant and the microbes. The symbiosis of Arabidopsis thaliana with the beneficial root-colonizing endophyte Serendipita indica is a well-studied system in which the co-cultivation of Arabidopsis roots with S. indica significantly promotes plant growth, including primary root elongation and lateral root formation. Although it has been suggested that this is due to a reprogramming of plant hormone levels, especially indole-3-acetic acid (IAA), to date, the molecular mechanism by which S. indica promotes plant root growth remains largely unknown. This study used comprehensive transcriptomics, reverse genetics, and confocal microscopy analyses to disclose the intricacies of auxin-related processes that affect root growth in the A. thaliana–S. indica interaction. The experiments revealed differential expression of a small group of genes encoding members of the GRETCHEN HAGEN 3 (GH3) protein family, especially GH3.5 and GH3.17, and of the PIN-FORMED (PIN) family of auxin transporters, especially PIN2. GH3s catalyse the inactivation of plant hormones, including auxin and salicylic acid, by conjugation with amino acids. PINs are transmembrane proteins responsible for the intercellular polar transport of auxin and for regulating auxin homeostasis at the intracellular level. Detailed phenotypic analysis of gh3 mutants and PIN and GH3 promoter reporter lines infected with S. indica confirmed our finding that PIN auxin transporters and GH3 acyl acid amido synthetases play a critical role in the studied plant-fungus interaction, thus broadening our knowledge about the role of auxin dynamics in plant-microbe interactions.

Interaction between leguminous plants and rhizospheric bacteria in a climate change context. Cynthia Meza[1,2], Francisca Valenzuela[3], Dayenu Olivares[2,4], Basilio Carrasco[3], Aparna Banerjee[5] (aparna.banerjee@uautonoma.cl). [1]Doctorado en Biotecnología Traslacional (DBT), Facultad de Ciencias Agrarias y Forestales de la Universidad Católica del Maule, Talca. [2]Centro de Biotecnología de los Recursos Naturales (CENBio), Facultad de Ciencias Agrarias y Forestales, Universidad Católica del Maule, Talca. [3]Centro de Estudios en Alimentos Procesados (CEAP), Talca. [4]Escuela de Ingeniería en Biotecnología, Facultad de Ciencias Agrarias y Forestales, Universidad Católica del Maule, Talca, Chile. [5]Instituto de Ciencias Aplicadas, Facultad de Ingeniería, Universidad Autónoma de Chile, Talca, Chile.
Abiotic stress is increasing day by day due to continuous global warming as well as climate change becoming one of the major causes behind the reduction in crop production. While anthropogenic impacts in a climate change context negatively impact native microbiome in an ecosystem, but plant-bacteria interaction plays an essential role in improving crop yield without using any chemical fertilizers. Our present study aimed to characterize the interaction between plant growth-promoting bacteria (PGPB) and their role in mitigating different abiotic stresses, viz. salinity, temperature, drought for local variety leguminous crops (Phaseolus vulgaris L.). Under salinity stress, the PGPB isolates indicated the production of stress components and cytoplasmic inclusion bodies. Visible root colonization of the bacteria has been observed in comparison to the control. More than 80% germination index was observed for all experimental setups of seed bacterization under temperature, drought, and salinity stress. A 10% tolerance of induced drought stress have been observed for the plants when germinated with the native PGPB and their consortium. Some of the plants also survived a temperature stress till 35 °C. The bacteria responded with good PGP traits that helped in the growth of healthy plants after the seed bacterization in final pot experiments. Additionally, the consortium and the plants treated with bacteria have demonstrated high production of photosynthetic pigments, antioxidant activity, and polyphenol content in the experimental setups which altogether have improved the growth of the plants. Our results indicate importance of native microbiome conservation, crop rotation with leguminous plants, health plant-bacteria interaction for improved agriculture.

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Biochemical and molecular insight of Antarctic Fungal endophytes improvement of strawberry crops under heat and drought stress. María Yáñez Ortega[1,2], Mario Moya[1], Sebastián Flores Valenzuela[1,2], Luis Morales-Quintana[3], Patricio Ramos[1,4]. [1]Plant-Microorganism Interaction Laboratory (PMIL), Instituto de ciencias biológicas, Universidad de Talca, Talca, Chile. [2]Facultad de Ciencias Agrarias y Forestales, Universidad Católica del Maule, Talca, Chile. [3]Multidisciplinary Agroindustry Research Laboratory, Instituto de Ciencias Biomédicas, Facultad Ciencias de la Salud, Universidad Autónoma de Chile, Talca, Chile. [4]Vicerrectoría de investigación y postgrado, Universidad Católica del Maule, Talca, Chile

Climate change poses a direct threat to food security, primarily due to global warming leading to heat-waves and drought that harm the growth and yield of agricultural crops. Research has demonstrated that a mutually beneficial relationship between plants and extremophile microorganisms plays a vital role in helping plants adapt to environmental stresses. In this study, *Fragaria x ananassa* plants were inoculated with to two endophytic fungi, *Penicillium chrysogenum* and *P. brevicompactum*, originally isolated from Antarctic plants. To simulate climate change conditions, various greenhouse tests were conducted under drought and high-temperature scenarios. The study assessed the biochemical physiological and molecular responses of both inoculated and non-inoculated plants in each treatment. The findings indicated that the inoculated plants showed a differential global molecular mechanism compared with non-inoculated control plants exposed to high temperature and water deficit. Physiological evaluations showed an improvement in water retention capacity, photosynthetic performance, and water use efficiency. Furthermore, the inoculated plants exhibited reduced levels of proline content and lipid peroxidation when exposed to drought and high temperatures. The research also revealed that the inoculation led to a modulation of antioxidant enzymatic activity, as well as increased levels of antioxidant chemical compounds and total antioxidant capacity. The results suggest the symbiotic relationship between Antarctic plants and microorganisms can alleviate the stress caused by water scarcity and high temperatures in crops leading to a modulation of the molecular mechanisms, which affects the physiological and biochemical performance in strawberry crops.

Funding: This work was supported by the project FONDECYT 1211057 and Anillo ATE220014

NEW MEMBERS AND INCORPORATIONS

Targeting Zeb1 and Zeb2 Transcription Factors for Leukemic Stem Cell Inhibition and Novel AML Therapeutic Discovery. Carlos Farkas[1] (cfarkas@ucsc.cl)*, Roberto Amigo[1], Andrew Cuddihy[2], Diego Cuevas[1], Adolfo Agurto[1], Antonia Recabal[3], Karina Oyarce[4], Joel Pearson[2], Katharina Haigh[2] and Jody Haigh[2]*. [1]Laboratorio de Ciencias Biomédicas, Universidad Católica de la Santísima Concepción, Chile. [2]CancerCare Manitoba, Winnipeg, Canada. [3]Departamento de Biología Celular, Facultad de Ciencias Biológicas, Universidad de Concepción, Chile. [4]Facultad de Medicina y Ciencia, Universidad San Sebastián, Concepción, Chile.

*Co-corresponding authors.

Acute myeloid leukemia (AML) is a clonal and heterogeneous disease marked by the accumulation of immature myeloid cells in the bone marrow and peripheral blood. One key challenge in AML treatment is the targeting of leukemic stem cells, as they are responsible for disease relapse. The aim of this project is to investigate the molecular mechanisms underlying AML pathogenesis and identify novel targets for drug development, with a focus on the role of the Epithelial-to-Mesenchymal (EMT) transcription factor ZEB2. We investigated the role of *Zeb2 in vivo*, using murine MLL-AF9-driven Acute Myeloid Leukemia (AML). Upon acute deletion of *Zeb2* at the single-cell level, we observed an expansion of neutrophil-like cells. These cells were notably absent in Cre-only MLL-AF9 mice, highlighting the significance of Zeb2 in regulating cellular compositions in this leukemia model. Notably, the repression of *Zeb2* led to the activation of *Cebpe*, a transcription factor critical for granulocyte differentiation, and *Cd177*, which plays a role in neutrophil activation. Such alterations could favor the proliferation of neutrophils at the detriment of other cell types, underscoring Zeb2's pivotal role in cell differentiation. UMAP dimensionality reduction analyses indicated distinct single-cell clusters between Cre-only and Zeb2-cre mice, which were consistent across biological replicates. Concurrently, there was a notable downregulation of the Hoxa5 gene and an upregulation of *Npm1*—genetic shifts linked with a more favourable prognosis and reduced cell proliferation in AML. Notably, after *Zeb1* deletion, a pronounced surge in *Zeb2* expression emerged, indicating a potential compensatory mechanism in AML. This underscores the potential of drugs targeting both *Zeb1* and *Zeb2* in AML management. In conclusion, our data highlight ZEB2's central role in AML progression and offer promising therapeutic avenues centered on ZEB2-targeted interventions. Further investigations are warranted to refine therapeutic strategies in AML.

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Nudt3 endo-polyphosphatase activity and its role in amyotrophic lateral sclerosis progression. Polett Garcés (polettgarces@gmail.com), Kevin Leiva, Brigitte van Zundert, Martín Montecino. Instituto de Ciencias Biomédicas (ICB), Facultad de Medicina, Universidad Andrés Bello, Santiago, Chile.

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the loss of motoneurons in the brain and spinal cord. We recently found that excessive release of inorganic polyphosphate (polyP), a ubiquitous negatively charged biopolymer, by mouse and human ALS astrocytes kills motoneurons. Here, we are studying the role and potential therapeutic applications of Nudt3, a recently described mammalian endo-polyphosphatase that degrades polyP in the presence of Zn2+. We propose that differential expression of this enzyme in wild-type and ALS astrocytes regulates polyP levels and motoneuron toxicity. We tested the activity of human recombinant Nudt3 with different sizes of synthetic polyP in the absence and presence of Zn2+ and other cations. Also, Nudt3 protein and transcript levels were analyzed in the spinal cord of ALS transgenic mice with mutations in SOD1 (mutSOD1) from asymptomatic stage to end-point. Nudt3-Zn2+ shows endo-polyphosphatase activity with synthetic long-chain polyP (130 and 700 Pi). Nudt3 transcript levels are decreased in the spinal cord of symptomatic mutSOD1 mice. Interestingly, Nudt3 protein levels are reduced from early asymptomatic stages till end-point. Nudt3-Zn2+ degrades long-chain polyP of similar size to that reported in the brain. Reduced Nudt3 levels in the spinal cord of symptomatic mutSOD1 mice correlate with high levels of polyP reported in astrocytes. To continue, we are evaluating whether the expression of this enzyme regulates polyP levels in primary astrocytes from mutSOD1 and control mice. Also, we are performing polyP-zymogram assays to identify *de novo* polyP regulating enzymes (polyphosphatases and polyP kinases) in primary astrocytes.

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Polyphosphorylation of Nucleolin in Amyotrophic Lateral Sclerosis Astrocytes: Identification of a Susceptible PASK Domain. Armando Amaro (a.amarosepulveda@gmail.com), Ignacio Leyton, Brigitte van Zundert, Martín Montecino. [1]Instituto de Ciencias Biomédicas (ICB), Facultad de Medicina, Universidad Andrés Bello, Santiago, Chile.

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease. We have found that ALS astrocytes, which kill motoneurons, have excessive inorganic polyphosphate (polyP) levels. PolyP can form covalent bonds with multiple proteins at specific lysine residues located within PASK domains, a process called polyphosphorylation (PPn). Studies in yeast revealed that PPn of nucleolin (Ncl) impacts its function and subcellular localization. An altered nucleolin distribution has been detected in ALS. Here, we tested whether Ncl-PPn occurs in ALS astrocytes (mutSOD1) and seeked to identify the responsible Ncl-PASK domain(s). Nuclear extracts obtained from control and mutSOD1 astrocytes were subjected to Nu-PAGE and Ncl was detected with a specific antibody. *In-silico* analysis was performed to identify theoretical Ncl-PASK domains. To determine which PASK domains in nucleolin are susceptible to PPn, each domain was cloned and fused to eGFP. Recombinant nucleolin PASK domains were incubated with synthetic polyP and subjected to Nu-PAGE. An electrophoretic Nu-PAGE mobility shift of Ncl was observed, particularly with nuclear extracts from mutSOD1 astrocytes compared to control astrocytes. While *in-silico* analysis identified that Ncl contains 5 potential PASK domains, *in vitro* studies with recombinant proteins revealed that only one PASK domain displayed an electrophoretic shift in the presence of synthetic polyP. Our study indicates increased Ncl-PPn in ALS astrocytes compared to control astrocytes. In-depth *in-vitro* investigations revealed a single PASK domain that can be polyphosphorylated. Currently, we are performing PASK motif mutagenesis to determine whether lysine is the specific amino acid responsible for Ncl-PPn.

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The Endoplasmic Reticulum master regulator protein BiP acts as a Ratchet molecular motor in translocation. Hilda M. Alfaro-Valdés[1±] (hilda.m.alfaro.v@gmail.com), Karina New[1±], Pablo S. Moya[2], Francesca Burgos-Bravo[4], Robert Lesch[3], Carolina Ramírez-Álvarez[1], Randy Schekman[3], and Christian A.M. Wilson[1]. [1]Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile. [2]Departamento de Física, Facultad de Ciencias, Universidad de Chile, Santiago, Chile. [3]Department of Molecular and Cellular Biology, Howard Hughes Medical Institute, University of California, Berkeley, USA. [4]California Institute for Quantitative Biosciences, Howard Hughes Medical Institute, University of California, Berkeley, USA.
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Post-translational protein translocation into the endoplasmic reticulum is mediated by the Sec61 complex and the auxiliary motor protein BiP (Immunoglobulin Binding Protein). The mechanism of this molecular motor has not been fully elucidated. Studies suggest that BiP could passively rectify movement of the translocating protein that occurs via Brownian motion (ratchet model) and/or an active mechanism of direct pulling (power stroke model). Here we have developed a novel methodology to study forces in bulk. This aims to determine if proteins with known unfolding forces can cross microsomal membranes by BiP-mediated unfolding of the protein. Constructs were engineered to include a signal peptide, unfolded Titin I27 domain (to allow entry into the microsomes), and the folded protein, and tested in translocation assays. First, a fully unfolded 2-Titin chimera protein construct was translocated into microsomes with an efficiency of 0.42%/min. While the Top7 chimera construct, with unfolding forces of around 20pN, displayed 0% translocation. Interestingly, the Calmodulin (CaM) chimera protein that unfolds at 7pN showed 0.02%/min translocation efficiency. This poor translocation efficiency in vitro suggests that BiP cannot exert sufficient forces to unfold the proteins and thus allow their translocation. In addition, the estimated mechanical work and output power turned out to be comparable to the local thermal energy, and orders of magnitude smaller than well-known power-stroke molecular motors, respectively. These results suggest that BiP does not actively contribute work to pull and unfold translocating substrates but acts as a ratchet-type molecular motor in post-translational protein translocation.
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Role of LOX-1 and oxLDL in the development of anti-androgen resistance in castration-resistant prostate cancer. Yerko Rivas, Marcela Mondaca, Javiera Sanzana , Felix Duprat, María Paz Castillo, Catalina Robles, Nery Jara, Romina Bertinat and Iván González-Chavarría (ivangonzalez@udec.cl). Laboratory of lipoproteins and cancer, Pathophysiology Department, Universidad de Concepción, Concepción, Chile.
LOX-1 is an important receptor for oxidated low-density lipoprotein (oxLDL). LOX-1 and oxLDL are pivotal factors in atherosclerosis, promoting important processes for the progression of this pathology, such as ROS generation, NF-kB activation, and the expression of IL-6, a STAT3 activator. In prostate cancer (CaP), upregulation of LOX-1 and a high concentration of oxLDL are associated with advanced clinicopathological stages. Furthermore, LOX1/oxLDL induces epithelial-mesenchymal transition, increasing angiogenesis and proliferation in CaP. Enzalutamide is an antagonist by androgen receptor (AR) used for castration-resistant prostate cancer (CRPC), but a high percentage of patients develop resistance to this drug. The decreased cytotoxicity is promoted in part by NF-kB and STAT-3 activation that induces the secretion of the pro-inflammatory program and the expression of AR and its splicing variant AR-V7, inducing intratumoral steroidogenesis. These markers are shared by abiraterone resistance, another anti-androgen used for CRPC, which inhibits androgen synthesis. However, the role of LOX-1 and ox-LDL on anti-androgen drug resistance has not yet been described. Our results demonstrated that oxLDL/LOX-1 increases ROS levels and activates NF-kB, inducing IL-6 secretion and the activation of STAT3 in human CRPC cells. Furthermore, oxLDL/LOX1 increases the expression of anti-androgen resistance markers, such as AR, AR-V7, and PSA, and intratumoral steroidogenesis markers, such as CYP17A1 and AKR1C1 expression. Thus, LOX-1/oxLDL decreases enzalutamide and abiraterone cytotoxicity in CRPC cells. Our investigation suggests that new factors associated with cardiovascular pathologies, such as LOX-1/oxLDL, may also promote important signaling axes for the progression of CRPC and its resistance against the few available anti-androgen drugs used for its treatment.

Unravelling copper toxicity mechanisms in *Staphylococcus aureus*. Javiera Norambuena[1,2] (javiera.norambuena@upla.cl) and Jeffrey Michael Boyd[1]. [1]Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, New Jersey, USA. [2]Departamento de Ingeniería para la Sostenibilidad, Facultad de Ingeniería, Universidad de Playa Ancha, Playa Ancha, Valparaíso, Chile.
Antimicrobial resistance is a global health emergency that has triggered an increase in demand to develop new drugs. Metals and metal ions have been used as effective antimicrobials for millennia, likely due to their ability to simultaneously inhibit multiple cellular processes. Copper (Cu) is a metal that has been used by humans as a prophylactic as well as a mechanism used by the human immune to aid bacterial killing or growth inhibition. Questions remain about how bacteria are toxified by new antimicrobial. This work was performed on the multi-drug resistant MRSA (Methicillin-Resistant *Staphylococcus aureus*) a world-wide global pathogen. To gain a better insight of how Copper (Cu) ions toxify cells, we created a strain that lacks the described Cu ion detoxification systems. We determined that Cu(II) exposure resulted in an increase in the concentrations of metabolites utilized to synthesize phosphoribosyl diphosphate (PRPP). PRPP is created using the enzyme phosphoribosylpyrophosphate synthetase (Prs), Increasing or decreasing expression of *prs* resulted in decreased and increased sensitivity to growth in the presence of Cu(II), and Cu(II) were able to inhibit this enzyme *in vivo* and *in vitro*. A nonbiased a suppressor screening was also conducted, this analysis revealed that mutations in *rpoB* and *rpoC* increased Cu(II)-tolerance. *rpoB* and *rpoC* encode for beta and beta' subunits of *S. aureus* RNA polymerase, respectively. We determined that this increase in Cu(II) tolerance was due to a decrease in intracellular protein precipitation in the suppressors strains. This work helps to understand how Cu(II) exerts toxicity in a pathogen of global concern.

Role of anthocyanase, polyphenol oxidase, and peroxidase enzymes in the anthocyanin degradation in cranberries and rosehip. Juan Román (jroman@udec.cl). Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile.

Anthocyanins, recognized as vital plant pigments abundant in cranberries and rosehips, are known to degrade during post-harvest processes due to various factors including light, temperature, and enzymatic actions. In these fruits, the precise enzymatic degradation mechanisms have been under-explored. Previous studies have elucidated the roles of anthocyanase (β-glucosidase), polyphenol oxidase, and peroxidase in the degradation process within fruits like grapes. However, a substantial knowledge gap persists regarding these enzymatic mechanisms in cranberries and rosehips. This line of research seeks to bridge this gap by hypothesizing that anthocyanin degradation in these fruits is predominantly regulated by anthocyanase, and that targeted enzyme inhibitors can mitigate this degradation.
To validate this hypothesis, the research undertakes a multi-faceted approach. The initial phase seeks to characterize and quantify post-harvest anthocyanin content, subsequently delving into the intricate kinetics of degradation under varied conditions, including differing pH, temperature, and the additions of specific modulators or inhibitors. A concluding element of this research is the exploration and optimization of post-harvest protocols to ensure maximal anthocyanin content.
The findings offer the potential to patent an innovative technology, preserving anthocyanins in cranberries and rosehips, with applications extendable to other anthocyanin-rich perishable fruits. From a global perspective, such advancements could elevate Chile's status, not just as a major exporter, but also as a leader in solutions that maintain the sensory and health qualities of fruits and foods.

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Gene-specific interaction of the long non-coding RNA MALAT1 and the Polycomb Repressive Complex 2 in human cancer cells. Michelle Tobar[1], Sebastián Fuentes[1], Cristopher Fierro[1], Daniela Nahuelquen[1], Francisco Quiroz[1], Andrea Matamoros[1], Martin Montecino[1], Patricio Cabane[2], Alvaro Elorza[1], Rodrigo Aguilar[1] (rodrigo.aguilar@unab.cl).
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The long non-coding RNA MALAT1 is one of the most overexpressed transcripts in several types of cancer cells. This RNA can bind directly to the DNA, collaborating with the epigenetic machinery for gene regulation. Among its partners, we have focused on the Polycomb Repressive Complex PRC2, a critical epigenetic regulator of gene expression during development and disease. In a model of human gastric cancer, we have conducted genome-wide analyses named CHART-seq and ChIP-seq to determine the genes targeted by the MALAT1 RNA and the PRC2 complex, respectively. The molecules occupy ~5,000 sites throughout the genome. Using antisense oligonucleotides to silence MALAT1, we found changes in PRC2 occupancy together with ~150 genes changing their expression. To complement the studies performed in cell lines, we conducted MALAT1 expression analyses in tissues obtained from cancer patients, finding increased expression in specific samples. Thus, our studies unveil MALAT1-dependent PRC2 sites and a set of MALAT1-controlled genes in gastric cancer. Additionally, our data show that, in certain patients, MALAT1 can be used as a biomarker.

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NLP7 is a central integrator of transcription networks in nitrogen signaling and drought stress in Arabidopsis thaliana. Nathan Johnson[1], Tomás C. Moyano[2], Viviana Araus[3], Jonathan Canan[1], Ji Huang[4], Carly Shanks[4], Samantha Frangos[4], Ariel Herrera[2], Francisca Blanco-Herrera[2], Gloria M. Coruzzi[4], Elena A. Vidal[1,3], José M. Alvarez[2,3] (jose.alvarez.h@unab.cl). [1]Centro de Genómica y Bioinformática, Facultad de Ciencias, Universidad Mayor, Santiago, Chile. [2]Centro de Biotecnología Vegetal, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago, Chile. [3]Agencia Nacional de Investigación y Desarrollo-Millennium Science Initiative Program, Millennium Institute for Integrative Biology (iBio), Santiago, Chile. [4]Center for Genomics and Systems Biology, New York University, New York, USA.

Plants constantly face multiple and often conflicting signals to balance growth and stress response. Although they are critical factors for plant growth and survival, the regulatory mechanisms that allow plants to integrate and respond to nitrogen (N) and drought signals still need to be discovered. Using a data-driven systems biology meta-analytical approach, we found a high overlap of N and drought-regulated genes across various experimental conditions showing opposite transcriptional patterns indicating they are largely contrary signals. Network-based analysis reveals common transcription factors (TFs) mediate N and drought gene responses. The NIN-LIKE PROTEIN 7 (NLP7), a master regulator of the N signaling pathway, was identified as a candidate TF to modulate N and drought interaction genes. Using a combinatorial experiment that varies N and drought levels simultaneously to quantify transcriptomes, we found that N dose impacts 55% of gene responses to drought, and water availability impacts 88% of N responses in Arabidopsis leaves. Most genes with altered expression in *nlp7* mutant plants respond to N and drought, indicating NLP7 has a specific role in integrating N and drought signals in leaves. Using a cell-based assay to capture direct TF regulation genome-wide in leaves, we demonstrated that NLP7 directly controls the expression of known N and drought TFs, such as LOB domain-containing protein 37 (LBD37), and RESPONSIVE TO DESICCATION 26 (RD26), respectively, which regulate downstream NLP7-dependent responses. Our validation studies show that NLP7-dependent pathways account for 85% of the interaction between N and drought. The *nlp7* mutant plants exhibit a drought-resistance phenotype influenced by the N availability in the soil. Taken together, our results reveal that convergent regulatory circuits underlie plant responses to the conflicting N and drought signals. This advances our understanding of how plants tilt the balance toward stress responses or growth regulation.

Resurrection of prestin ancestors shed light on the emergence of its area-motor activity.

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Prestin, a member of the SLC26 family of transporters, functions as an area-motor crucial for the generation of electromotility (EM), a fundamental process for the amplification and tuning of auditory signals. Strikingly, prestin only plays this role in mammals. In non-mammals, it acts as an anion transporter without piezoelectric activity. Studies aimed at explaining the origin of these differences found at least two regions of interest in the transmembrane domain (TMD). However, to date, no structural explanation has been provided. Furthermore, a region of high variability among members of the SCL26 family has been overlooked: The IVS on the STAS domain. This disordered region, which is absent from all SLC26 structures available, shows unique sequence features of mammalian prestin that could be related to EM generation. In this study, we combine phylogenetic, resurrection of ancestral prestin representatives and structural modeling to offer a comparative analysis of prestin evolution from a structural point of view and shed light on how its unique sequence features may play a role in the generation and tuning of EM. Our results suggest that sequence/structural differences in TMD are related to changes in the interactions between helices, which may hinder the conformational transition that would lead to the outward open state. Regarding the IVS, our results suggest that this region may directly interact with residues that form the entrance of the intracellular cavity of the adjacent subunit and thus modulate the response to voltage or frequency changes.

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Steamer Like Element-1 antisense RNA harbor an Internal Ribose Entry Site. Leandro Fernández-García(leandrofg1990@gmail.com), Gloria Arriagada. Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad Andrés Bello, Santiago, Chile.

LTR-retrotransposons are transposable element characterized by the presence of long terminal repeats (LTRs) directly flanking an internal coding region. They mobilize through reverse transcription of their mRNA and integration into another location. LTRs contain sequences that regulate transcription and translation. We identified an LTR-retrotransposon in the genome of *Mytilus chilensis* named Steamer-like element-1 (SLE-1). Both DNA strands have putative open reading frames in KOZAK consensus and with poly-adenylation signals and transcriptome analysis showed the presence of RNA derived from both strands of SLE-1. To verify if SLE-1 generate sense and antisense mRNA, strand specific cDNA synthesis and qPCR was performed. This assay confirmed that both RNAs are generated in the animals. To analyze whether these RNAs can function as messengers, luciferase reporters commanded by the untranslated regions (UTR) of SLE-1 were generated. For both SLE-1 5´UTR translation can initiate canonically, but only antisense SLE-1 5´UTR can initiate translation when eIF4E-eIF4G interaction is disrupted, suggesting the presence of an internal ribosomal entry site (IRES) on the antisense 5´UTR. To test this possibility bicistronic reporters were generated. When bicistronic mRNA were transfected in cells no IRES activity was detected, but when cells were transfected with the plasmids encoding the bicistronic mRNA, the 5´UTR IRES activity is revealed. These results indicate that SLE-1 IRES located in the antisense transcripts requires a nuclear experience to be functional, trough the recruitment of RNA binding protein. In this work, we show for the first time an antisense RNA with IRES activity of a retrotransposon in mollusk.

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Spatial long-range correlations in coupled and bistable gene networks. Kevin Simpson[1,2], Alfredo L'Homme[1,2], Alejandro Aravena [1,2], Isaac Nuñez [1,2], Maria L. Cordero[3], Tim Rudge[4], Juan Keymer[5, 6], Fernan Federici[1,2] (ffederici@bio.puc.cl). [1]ANID – Millennium Science Initiative Program, Millennium Institute for Integrative Biology (iBio), Santiago, Chile. [2] Institute for Biological and Medical Engineering, Schools of Engineering, Medicine and Biological Sciences, Pontificia Universidad Católica de Chile. [3] Departamento de Física, FCFM, Universidad de Chile, Santiago, Chile. [4] Interdisciplinary Computing and Complex Biosystems, School of Computing, Newcastle University, Newcastle, United Kingdom. [5] Institute for Advanced Studies, Shenzhen X-Institute, Shenzhen, China. [6] Department of Natural Sciences and Technology, Universidad de Aysén, Coyhaique, Chile.

Developing a common theoretical framework to study the scaling laws of gene patterns remains a fundamental problem in biology. Gene networks are able to self-organize into short and long-range correlation and anticorrelation patterns through mechanisms that differ widely across different systems, from bacterial films to eukaryotic tissues. To uncover generic principles of this phenomenon, we use a theoretical framework of the Ising model from statistical mechanics, computer simulations and synthetic gene networks (SGNs). We show how SGNs with two states, which are positively or negatively coupled across cells, are able to exhibit patterns of long-range correlations with power-law scaling behavior or anti-correlations, respectively. These patterns, resembling ferromagnetic and anti-ferromagnetic configurations of the Ising model near a critical state, maintain their scaling properties upon changes in growth rate and cell shape. The scaling behavior in both simulated and in vivo ferromagnetic colonies is in close agreement with the cluster size distribution near the critical percolation threshold, at which the system moves from a regime of only localized short-range patches to one with clusters that span the entire system. We also apply Finite-Size Scaling (FSS) analysis to define critical exponents and the universality class. We later use spatially-coupled differential equations with the aim of defining a control parameter that enables the search of these universal exponents in growing colonies. These theoretical and experimental results suggest that positively coupling genetic switches can pose cell populations near the critical point of a phase transition in which far regions in the colony are correlated.

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Temperature, voltage and potassium dependence of charybdotoxin binding to Shaker Potassium Channels, a case of lock and key equilibrium. Nieves Navarro[1], Francisca Salas[2], Horacio Poblete[2], David Naranjo[1](david.naranjo@uv.cl). [1]Instituto de Neurociencia, Universidad de Valparaíso, Valparaíso, Chile. 2 CBSM, Universidad de Talca, Talca, Chile.

As a classical example of the lock-key binding mechanism, Charybdotoxin (CTX), a toxin from the scorpion *Leiurus quinquestriatus*, binds to the external pore entrance of voltage-gated Shaker K-channels with extreme simplicity. CTX binding just plugs the pore preventing K^+ flow, without significant conformational impact on either protein. K-channels are membrane proteins specialized in passive potassium transport. When expressed in *Xenopus* oocytes K-channel activity can be recorded as an electrical current (carried by K^+ moving across the membrane) with the two-electrode voltage-clamp technique. CTX application at 5 nM produces a significant and characteristically voltage dependent reduction of the current. Interestingly, both, CTX association and dissociation rates are sensitive to external K^+ in a unexpected way: 1) At room temperature, the association rate in ~100 mM external K^+ is 2-fold higher than in ~100 mM external Na^+ in the perfusion bath. 2) The dissociation rate is enhanced in 100 mM external K^+, revealing the existence of dislocated binding states (*wobbling*). We investigated the effects of K^+ ions, the electric field, and temperature on the CTX binding equilibrium with the aim of identifying the intermediate events leading to the CTX association and dissociation. The dissociation rate showed more temperature, voltage, and potassium sensitivity, suggesting that during CTX-wobbling, external K^+ accessed the blocked channel pore. Accordingly, voltage applications in MD simulations show wobbling with sporadic permeation events. Thus, as proposed 35 years ago, the electric field can force potassium ions within the pore in the direction of the external entry, electrostatically destabilizing CTX.

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Pharmacological Inhibition of the HSC70/LAMP2A Complex for the Development of Potential Anticancer Drugs. Gonzalo Núñez[1,3](gonzalo.nunez@udd.cl), Carlos Lagos[2], Tomás Perez-Acle[3], Iván Afaro[1]. [1]Laboratorio de Fisiología Celular, Universidad del Desarrollo. [2]Escuela de Química y Farmacia, Universidad San Sebastian. [3]Computational Biology Lab, Fundación Ciencia & Vida

Chaperone-Mediated Autophagy (CMA) is a process in which proteins bearing the KFERQ motif are translocated through the lysosomal membrane for subsequent degradation. The primary proteins responsible for this process are HSC70, a chaperone responsible for motif recognition, and LAMP2A, a transmembrane protein that interacts with HSC70 and forms the pore to facilitate the internalization of the target protein. It has been reported that CMA is highly activated in various types of cancer, both in tissues and cell cultures, compared to their non-cancerous counterparts, and furthermore, it provides protection against hypoxia and nutrient deprivation. Inhibition of this pathway has also been shown to block the growth and metastatic properties of tumors in animal models. In this study, we propose the development of drugs with the ability to inhibit the complex formation as a potential cancer treatment. In this investigation, comparative modeling and molecular dynamics were employed to obtain the most probable conformations of HSC70 and LAMP2A. Subsequently, a model of the complex was constructed through docking, inserted into a lysosomal membrane, and subjected to molecular dynamics simulations to evaluate its stability. Finally, upon achieving a stable complex, docking was performed on the interaction region of HSC70 using the MCULE and NCI databases, encompassing over 3 million compounds. The resulting outcomes were subsequently filtered based on ADME properties, yielding several molecules with a high probability of inhibition, of interest for an analog program, and thus representing a novel therapeutic option. Of the compounds that have been tested so far, 3 have shown activity in viability assays on the breast adenocarcinoma cell line MCF-7 in the micromolar range. Among the evaluated compounds so far, three have demonstrated activity in viability assays conducted on the breast adenocarcinoma cell line MCF-7, within the micromolar concentration range.

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Glycosylated delphinidins as a potential sensitizing compound to temozolomide treatment in glioblastoma cells by regulating NF-κB/ MGMT and COX-2/MDR1 signaling. Carrillo-Beltrán Diego[1,2,3](diego.carrillo@uach.cl), Fabres Karen[2], Cuevas Constanza[1,2], Silva Pamela[1], Inostroza Sebastian[2], Quezada-Monrás Claudia[1,3]. [1]Laboratorio Biología Tumoral, Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Valdivia 5110566, Chile. [2]Laboratorio Virología Molecular, Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Valdivia 5110566, Chile. [3]Millennium Institute on Immunology and Immunotherapy, Facultad de Ciencias, Universidad Austral de Chile,Valdivia 5110566, Chile.

Glioblastomas (GB) are a global health problem, as it is the most common and aggressive malignant brain tumor. Temozolomide (TMZ) is the leading and most effective clinically used drug to treat GB. However, approximately 55% of GB patients are resistant to TMZ. The main mechanisms of TMZ resistance are mediated by the direct response of the methyl guanine methyl transferase (MGMT) enzyme repair system, the increase of a population of highly chemoresistant cells called glioblastoma stem cells (GSCs), and the upregulation of some ATP-binding transporters such as MDR1. Downregulation of the NF-κB/MGMT and COX2/MDR1 pathways has been suggested as an excellent strategy to increase the sensitivity of tumor cells to TMZ. In this regard, glycosylated delphinidins are potential negative regulators of COX-2 and NF-κB. Thus, we hypothesize that glycosylated delphinidins downregulate NF-κB/MGMT and COX2/MDR1 signaling, thereby inducing GB cell sensitization to TMZ. We initially analyzed the effect of glycosylated delphinidins on NF-κB/MGMT and COX2/MDR1 signaling in GB cells. Subsequently, we characterized the regulation of NF-κB/MGMT and COX-2/MDR1 signaling in the presence of glycosylated delphinidins in GB cells. Next, we evaluated the combined effect of TMZ with glycosylated delphinidins on GB cell viability and apoptosis. Our results indicate that glycosylated delphinidins regulate the expression of MGMT and MDR1 by downregulating the NF-κB pathway and COX2 activity, respectively. Finally, we observed increased TMZ sensitization in GB cells when treated with glycosylated delphinidins. We suggest glycosylated delphinidins as a possible adjuvant to temozolomide chemotherapy, as they reduce chemoresistance by regulating MDR1 and MGMT.

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Biochemical characterization of functional innovation in the vitamin kinase family: emergence of pyridoxal kinase activity in ThiD2- PLK/HMPK enzymes. Nicolás Fuentes-Ugarte (nicolas.fuentes@ug.uchile.cl), Victoria Guixé and Víctor Castro-Fernández. Laboratorio de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Chile, Santiago, Chile.

The ATP-dependent vitamin kinase family is involved in vitamin B1 and B6 biosynthesis by phosphorylating different substrates. Within this family, two enzymes can perform the phosphorylation of hydroxymethyl-pyrimidine (HMP) to hydroxymethyl-pyrimidine-phosphate (HMP-P). First, a bifunctional pyridoxal kinase encoded by a *pdxK-like-ThiD* gene (ThiD2-PLK/HMPK) that can phosphorylate HMP to HMP-P and can also phosphorylate pyridoxal (PL) to pyridoxal-5-phosphate. Second, a hydroxymethyl-pyrimidine-phosphate kinase encoded by the *thiD* gene (ThiD-HMPPK) that phosphorylates HMP to HMP-P and consecutively phosphorylates HMP-P to produce hydroxymethyl-pyrimidine-diphosphate (HMP-PP). Phylogenetic analysis indicates that ThiD2-PLK/HMPK enzymes diverged from ThiD-HMPPKs, suggesting that the HMP-P kinase activity would have been lost during evolution while the PLK activity appeared as an evolutionary novelty in the ThiD2-PLK/HMPK group. In this work, we reconstructed ancestral sequences from these enzyme families, which were expressed in recombinant form, purified and characterized. Biochemical characterization shows that the last common ancestor of ThiD-HMPPK can only phosphorylate HMP and HMP-P, but an intermediary ancestor between ThiD-HMPPK and ThiD2-PLK/HMPK enzymes (named ancC), gain the capability of phosphorylate PL besides the phosphorylation of HMP and HMP-P. Along the evolutionary pathway, the last common ancestor of ThiD2- PLK/HMPK enzymes can phosphorylate PL, HMP, and HMP-P, but there is a remarkably change in substrate specificity, being PL phosphorylation the enzymatic activity with the highest catalytic efficiency. These results suggest that the functional optimization of PL phosphorylation was concomitant with the loss of HMP-P phosphorylation, highlighting that a functional evolutionary novelty can co-exist with the ancestral function along the evolutionary pathway of an enzyme family.

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Palmitic acid disrupts PKD2/BECN1 complex formation leading to autophagy inhibition in hypothalamic neurons. Camila García-Navarrete[1,2,3] (camila.garcia.n@ug.uchile.cl), Catalina Kretschmar[3], Daniel Peña-Oyarzún[3,4], Eugenia Morselli[5], Valentina Parra[1,2], Alfredo Criollo[2,3]. [1] Department of Biochemistry and Molecular Biology, Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile. [2] Advanced Center for Chronic Diseases (ACCDiS), Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile. [3] Instituto de Investigación en Ciencias Odontológicas, Facultad de Odontología, Universidad de Chile. [4] Interdisciplinary Center for Research in Territorial Health of the Aconcagua Valley (CIISTe Aconcagua), School of Medicine, Faculty of Medicine, San Felipe Campus, Universidad de Valparaíso, Chile. [5] Department of Basic Sciences, Faculty of Medicine and Sciences, Universidad San Sebastián, Santiago, Chile.

The primary cilium is an evolutionary conserved microtubule-based organelle that senses extracellular stimuli that leads to the activation of different signaling pathways to maintain cellular homeostasis. Recent studies have demonstrated that the primary cilium regulates autophagy, an intracellular process that recycles proteins and organelles. Interestingly, our group has previously shown that polycystin-2 (PKD2), a protein enriched in the primary cilium, not only is required and sufficient for autophagy induction, but also interacts with Beclin-1 (BECN1), a critical protein of the autophagy core machinery. On the other hand, palmitic acid (PA) is a saturated fatty acid that accumulates in the hypothalamus of obese subjects where it also blocks autophagy flux. However, the mechanism by which PA blunts autophagy in hypothalamic neurons, is unknown. We hypothesized that PA disrupts the ciliary complex PKD2-BECN1, leading to autophagy flux inhibition in hypothalamic cells. Through confocal fluorescence microscopy, we analyzed the localization of PKD2 and BECN1 at the primary cilium; and by using specific ciliary markers, we found that both PKD2 and BECN1 co-localize in this organelle. In addition, co-immunoprecipitation assays revealed that PKD2 and BECN1 are forming a protein complex, which is lost following PA treatment. Moreover, PA inhibits autophagy, and this was modulated by PKD2. In conclusion, our data suggest that PKD2 is forming a complex with BECN1 in the primary cilium of hypothalamic neurons, which is modulated by PA to block autophagy. Thus, our work could be key for the development of new treatments for obesity-related diseases that involve hypothalamic neurons.

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The ER-phagy receptor FAM134B is targeted by *Salmonella* Typhimurium to promote infection. Damián Gatica[1](dgaticam@uottawa.ca), Reham Alsaadi[1], Rayan El Hamra[2], Dikchha Rijal[2], Subash Sad[2] & Ryan Russell[1]. [1]Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, ON, Canada [2]Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, ON, Canada

The Endoplasmic Reticulum (ER) is a dynamic intracellular organelle involved in protein and lipid synthesis, post-translational modifications, and trafficking. ER stressors, such as protein aggregates can be toxic for the cell, thus different pathways such as the unfolded protein response and autophagy are necessary to maintain ER homeostasis. Autophagy is a conserved degradation pathway that involves the recycling of multiple cellular components in response to different types of stress. Selective autophagic degradation of the ER is termed ER-phagy, during which portions of the ER are sequestered, transported, and degraded in lysosomes. ER-phagy controls ER size, activity, and the removal of protein aggregates or organelle damage, thus restoring ER homeostasis. ER-phagy can also be stimulated by pathogen infection, which contributes toward the innate antibacterial response. However, the link between ER-phagy and bacterial infection remain poorly understood, including the mechanisms pathogens have evolve to evade the effects of ER-phagy. We identified a novel mechanism of pathogen-mediated inhibition of ER-phagy. Specifically, we found *Salmonella enterica* serovar Typhimurium inhibits ER-phagy by specifically targeting the ER-phagy receptor FAM134B. We show that the *Salmonella* effector SopF prevents FAM134B oligomerization, which is required for efficient ER-phagy. Whereas ER- phagy inhibition increases intracellular *Salmonella* content, FAM134B overexpression and ER-phagy activation resulted in increased bacterial clearance. Furthermore, FAM134B knock-out mice infected with *Salmonella* presented increase bacteria levels in the spleen, colon and feces when compared to infected wild-type controls. Overall, our results provide new mechanistic insight into the interplay between ER-phagy and bacterial infection.

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Generation and characterization of a novel fully human antibody towards the MICA protein, a therapeutic target in Gastric Cancer.

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Gastric cancer (GC) presents a significant public health challenge due to its high mortality. Despite current efforts in chemotherapy, effectiveness remains limited, prompting the search for new therapeutic strategies. In this context, the MICA protein has been proposed as a potential target in immuno-oncology, given its involvement in immune evasion through its interaction with NKG2D. This study focuses on the development of a fully human antibody targeting MICA, with the aim of enhancing the immune response against GC. Using phage library technology, we selected a single-chain variable fragment (scFv) against MICA and then the AcHuαMICA antibody was generated. Its production was carried out in CHO-S cells and purified by FPLC. The interaction between the antibody and MICA was assessed *in vitro* using FC to measure binding in gastric tumor cells and transfected cells. The antibody affinity and its ability to block the interaction between MICA and NKG2D was performed by ELISA. In addition, *in vivo* binding capacity and biodistribution was analyzed using NOD *scid gamma* mice by IVIS-ILUMINA imaging, after establishing a tumor with MICA transfected B16F10 cells. The results demonstrated that AcHuαMICA neutralized the soluble form of MICA *in vitro* and exhibited superior affinity to the scFv against MICA. In addition, its ability to bind to various MICA variants and its accumulation at the tumor site in the murine model was also demonstrated. In conclusion, properties that support continuing with the development of a new drug aimed at improving the treatment of GC or another tumor that expresses MICA.

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Extracellular vesicles isolated from the serum of patients with *Helicobacter pylori* contain circulating Hp-OMVs. María Fernanda González C.[1,2](mfe.gonzalez@gmail.com), Marcelo J Kogan [2,3], Alejandro Corvalán [2,4], Manuel Valenzuela-Valderrama[2,5],Andrew F.G. Quest[1,2#] and Lisette Leyton[1,2#]. [1]Laboratorio de Comunicaciones Celulares, Centro de Estudios en Ejercicio, Metabolismo y Cáncer (CEMC), Programa de Biología Celular y Molecular, Facultad de Medicina, Universidad de Chile, Santiago, Chile. [2]Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Centro Avanzado para Estudios en Enfermedades Crónicas (ACCDIS), Santiago, Chile. [3]Departamento de Química Farmacológica y Toxicológica, Universidad de Chile, Santos Dumont 964, Santiago 8380494, Chile. [4]Departamento de Hematología-Oncología, Facultad de Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile. [5]Laboratorio de Microbiología Celular, Instituto de Investigación y Postgrado, Facultad de Ciencias de la Salud, Universidad Central de Chile, Santiago 8330546, Chile.

Helicobacter pylori (Hp) is an extremely prevalent bacterium associated with gastric diseases and an increased risk of developing gastric cancer. Recently, stomach infection with *Hp* was found to correlate with the presence of extra-gastric pathologies. The mechanisms by which *Hp* induces or exacerbates extra-gastric diseases are unknown. However, nanovesicles released from the outer membrane of *Hp* (*Hp*-OMVs) could enter the bloodstream and reach different tissues, promoting such diseases. There are limited reports on the detection of OMVs in human body fluids, which may be due to difficulties in isolating OMVs versus host cell-derived Extracellular Vesicles (EVs). Here, we isolated EVs from the serum of 20 Chilean patients infected or not with Hp. The EVs obtained in both patient groups were of similar sizes and concentrations. Then, we evaluated by western blotting the presence of exosome markers and *Hp*-urease. We detected the presence of *Hp*-urease in EVs from the serum of roughly 50% of the patients infected with *Hp* but none in non-infected patients. The presence of *Hp*-urease in EVs from the serum of *Hp*+ infected patients suggests that most likely these EVs are *Hp*-OMVs. *Hp*-OMVs, found in the serum of Hp+ patients indicate that such vesicles cross the stomach epithelial barriers and reach the bloodstream. Therefore, systemically OMVs may represent the mechanism by which *Hp* is connected to extra-gastric diseases. These observations open up the possibility of developing a new non-invasive diagnostic methodology, which could allow identifying patients with an elevated risk of developing extra-gastric health problems.

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Exploring the Adaptation Strategies of *Cistanthe longiscapa* in the Arid Atacama Desert. Ossa P.[1,2,3], Moreno A.[1], Orellana D.[4], Toro M.[1], Carrasco-Valenzuela T.[1], Riveros A.[1,2], Meneses C.[2,5,6,7], Nilo-Poyanco R.[8], Orellana A[1,2] (aorellana@unab.cl). [1]Centro de Biotecnología Vegetal, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago, Chile. [2]Instituto Milenio, Centro de Regulación del Genoma, Santiago, Chile. [3]Escuela de Veterinaria, Facultad de Ciencias, Universidad Mayor, Santiago, Chile. [4]Escuela de Agronomía, Facultad de Ciencias Agronómicas, Pontificia Universidad Católica de Valparaíso, Chile.[5]Departamento de Fruticultura y Enología, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Santiago, Chile. [6]Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile. [7]ANID - Millennium Science Initiative Program - Millennium Nucleus for the Development of Super Adaptable Plants (MN-SAP), Santiago, Chile.[8]Escuela de Biotecnología, Facultad de Ciencias, Universidad Mayor, Santiago, Chile.

Studying how plants thrive and exhibit diverse traits in different environments is critical to understanding adaptation. *Cistanthe longiscapa*, a distinctive desert plant that utilizes Crassulacean Acid Metabolism (CAM), thrives in the arid Atacama Desert. This study examined three sites with different soil types and rainfall levels. We assessed traits such as nocturnal leaf acid accumulation, $\delta^{13}C$ isotope ratio, photosynthetic pigments, carbon to nitrogen ratio, leaf mass per area (LMA), and leaf succulence (SWC) at each site. The observed trait differences were consistent with the different precipitation levels at each site. Principal component analysis (PCA) revealed strong relationships between certain variables and the geographic distribution of sampling sites. In addition to studying ecophysiology, we analyzed the leaf transcriptome of *Cistanthe longiscapa*. We assembled and compared differentially expressed genes. RNA-seq data from 18 libraries from different locations and times of day resulted in 88,770 unique transcripts and 37,253 predicted proteins. PCA was used to identify gene expression patterns associated with location and collection time. Differential gene expression analysis highlighted notable changes in transcripts such as LHY transcription factor, NADP-dependent malic enzyme, and P5CS. Examination of enriched functions revealed metabolic differences between sites, with unique processes enriched at each site. This study shows how *Cistanthe longiscapa* responds ecophysiologically and transcriptomically to different environmental conditions. These findings provide valuable insights into the plant's adaptation mechanisms in the harsh Atacama Desert.

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Translation initiation of the minor isoform of DeRep, an Endogenous Parvoviral protein expressed in *Octodon degus.* Pablo Lobos[1] (p.lobosvila@uandresbello.edu), Leandro Fernández-García[1] and Gloria Arriagada[1]. [1]Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad Andrés Bello, Santiago, Chile.

Endogenous parvoviral elements (EVPs) are genomic sequences highly represented in mammalian genomes, that derived from ancestral parvoviral infections of germ line or early embryos. We recently showed in *Octodon degus* (degu) that the locus *EPV-Dependo.43ODegus* derived from the ancestral parvoviral replicase with an intact open reading frame (ORF), is expressed as a protein in degu. By western blot assays, using two different antibodies we were able to detect a protein corresponding to the entire putative ORF (DeRep) and a smaller protein in different degu organs. By sequence analysis of DeRep coding sequence we revealed the presence of two in frame downstream start codons that could serve as translation start sites for the smaller protein. The coding sequence for DeRep was *in vitro* transcribed and translate, using the two anti DeRep antibodies, we again detected the full-length protein ($DeRep_L$) and the smaller protein (DeReps), which confirm that from this mRNA two different proteins can be expressed. To study the translation mechanism of $DeRep_L$ and $_S$ the plasmid pDeRepRLuc was generated containing the DeRep sequence fused to the Renilla luciferase coding sequence (RLuc). When NIH3T3 are transfected with plasmids encoding DeRepRLuc and Foot-and-mouth disease virus (FMDV) leader protease, we observed that this mRNA translation is sensitive to eIF4E-eIF4G interaction disruption and depends on 5´-cap structure. This result suggest that $DeRep_S$ is synthesized from a non-canonical cap-dependent translation initiation mechanism, such as leaky scanning. Mutation of the main AUG in pDeRepRLuc still shows luciferase activity, this strongly suggest that leaky scanning is the mechanism of $DeRep_S$.

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X-ray crystallography and ligand specificity of the AMP allosteric site of Bifunctional PFK/GK Enzymes from Methanogenic Archaea. Sixto M. Herrera (sixto.morales@ug.uchile.cl), Andrés Urrutia Santana, Daniela Malavé, Nicolás Fuentes-Ugarte, Erwin Quiroga, Gabriel Vallejos-Bacceliere, Víctor Castro-Fernandez, Victoria Guixé. Laboratorio de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Chile.

In the glycolytic pathway of Archaea, the glucokinase (GK) and phosphofructokinase (PFK) reactions were initially reported as non-regulated. However, our group recently reported and described the allosteric AMP activation in bifunctional PFK/GK enzymes from the order *Methanococcales*. In this work, we employed X-ray crystallography to solve, at a resolution of 1.6 Å, the atomic structure of the bifunctional PFK/GK enzyme from *Methanothermococcus termolitotrophicus* (MttPFK/GK) in complex with AMP at the allosteric site. Using this high-resolution structure, we performed 400 ns of all-atom molecular dynamics simulations, assessing the stability of the interactions involving the AMP bound to the allosteric site. This allows us to identify the amino acid residues that have the highest interaction energy with AMP through MMPBSA and the linear interaction energy algorithms. Additionally, we evaluated the ligand specificity evolution of the allosteric site, by a kinetic comparative study between the MttPFK/GK and the reconstructed ancestor of the PFK/GK enzymes from *Methanococcales* order (ancM). We assayed different monophosphate nucleotides such as AMP, cyclic-AMP, deoxyAMP, IMP, GMP, TMP, as well as adenosine on their ability to activate the GK and PFK activities of the extant and ancestral enzyme. The results revealed a high specificity of the allosteric site for AMP, with the nitrogenous base ring and phosphate playing crucial roles as determining factors for allosteric activation.

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Polypharmacology and Drug Repurposing for Atrial Fibrillation: A Structural Biology Approach. José Carlos Estanislao Márquez Montesinos[1] (jcemarquezm@gmail.com), Yuliet Mazola Reyes[1], Luciano Peña[1], Aytug K. Kiper[2], Kirsty Vowinkel[2], Marlene A. Komadowski[2], Susanne Rinné[2], Niels Decher[2], Wendy González Díaz [1,3]. [1]Center for Bioinformatics, Simulation and Modeling (CBSM), Universidad de Talca, Chile. [2]Institute of Physiology and Pathophysiology, Vegetative Physiology, Philipps-University Marburg, Marburg, Germany. [3]Millennium Nucleus of Ion Channels-Associated Diseases (MiNICAD), Chile.

Atrial fibrillation (AF) is the arrhythmia with the highest global prevalence, and there is a growing tendency towards increased hospitalizations in both Chile and the rest of the world. This condition arises from the alteration of the behavior of atrial ion channels, resulting in the degeneration of cardiac rhythm. Given the complex nature of AF as a physiological disease, the polypharmacology (PP) approach could offer a potential solution. The PP paradigm focuses on discovering a molecule capable of exerting a biological effect on several selected main targets of the same pathology, referred to as a multi-target directed ligand (MTDL). It is understood that correcting the cardiac rhythm in the context of AF involves the blocking of atrial-selective potassium channels Kv1.5 and TASK-1, as well as the cardiac sodium channel Nav1.5. An example of MTDLs are the local anesthetics (LAs) such as lidocaine, bupivacaine al ropivacaine. They block the atrial ion channels Kv1.5, TASK-1and Nav1.5. The objective of this research was to identify a MTDL specifically blocking these cardiac ion channels. To achieve this goal, a bioinformatic protocol was developed. Molecular Dynamics Simulation frames of the three channels were employed to analyze and compare the physicochemical and geometrical features of LAs binding sites (BSs). Subsequently, representative frames and groups of equivalent residues among the BSs were extracted. This information guided a virtual screening of FDA-approved drugs containing the pharmacophore of the LAs. Through this process, two hits (WE1 and WE2) were identified that demonstrated binding affinity to all three channels while interacting with equivalent residues within their respective BSs. This interaction pattern suggests a potential polypharmacological behavior. At a concentration of 10µM, WE2 exhibited a percentage of blockage in the same order of magnitude across the three channels. Importantly, no activity was observed in other cardiac channels such as hERG, Kir2.1, and Kv4.3, confirming WE2's role as a MTDL. Furthermore, as WE2 is an FDA-approved drug, a repurposing strategy could be employed in the near future for the treatment of AF. Keywords: Multi-target; drug promiscuity; druggable binding site; Local anesthetics; Nav1.5; Kv1.5; TASK-1; binding site comparison; polypharmacology, Drug Repurposing.

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Mass photometry as novel approach to study oligomerization and structure-function relationship of immunoglobulin binding protein (BiP). Karina New[1] (karina.knew@gmail.com), Roi Asor[2], 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

Human immunoglobulin binding protein (BiP) self associates into multiple oligomeric species that may affect its activity, in particular interaction with its protein client. Mass photometry (MP) assays can be employed to study the stability of BiP monomers in solution and their tendency to assemble into higher-order oligomers. When performed as a function of substrate and nucleotides, binding parameters such as affinity and dissociation kinetics can be eluded from mass photometry studies. MP studies reveal similar BiP dimer:monomer ratios in solution in absence of nucleotides, and presence of ADP or ATP-g-S (0.13, 0.21 and 0.11, respectively). However, when ATP is added this drops to 0.03. This indicates that while ADP does not affect the interactions between BiP subunits, a monomer stabilization effect occurs upon ATP binding. This effect of ATP was studied in detail and it was determined that the dissociation constant (KD) of ATP with BiP is 0.1 μM, similar to that determined by other techniques such as nano-rheology (doi*:10.1002/pro .3432*). Nucleotide dependent oligimerisation of BiP was also studied via MP in the presence of small peptide binding targets of BiP. Two heptomeric peptides previously shown to bind to BiP in solution (*doi:10.1016/0092-8674(93)90492-9, doi:10.1038/nsmb.1970*) with KD's of ~ 15uM are added to experiments of all nucleotide conditions (absence, ADP, ATP and ATP-g-S). KD's of nucleotides in the presence and absence of substrate are compared, shedding light on the relationship between the allosterically linked substrate and nucleotide binding domains of BiP. Substrate KD's are compared to previous findings, validating MP approaches.
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On the role of local unfolding in catalysis modulation. M Agueda Placenti[1,2] (aguedaplacenti@gmail.com), Santiago Martinez-Gache[1,2], Mauricio Báez Larach [3], Rodolfo M Gonzalez Lebrero [1,2], F Luis Gonzalez Flecha[1,2], Ernesto A Roman [2,4]. [1]Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina. [2]Instituto de Química y Fisicoquímica Biológicas, Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina. [3]Laboratorio de Bioquímica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile. [4]Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina.

P-ATPases constitute a family of membrane proteins that couple ATP hydrolysis to the transport of solutes across biological membranes, forming a phosphorylated intermediate during their reaction cycle. These proteins share a transmembrane domain through which the substrate is transported. Also share cytoplasmic domains that consists of a catalytic modulator domain (A or "actuator") and an ATP binding and hydrolysis domain (ATPBD or “ATP binding domain") comprised by the N ("nucleotide binding") and P ("phosphorylation") subdomains. ATPBD crystallographic structures reveal different conformational states related to open-closed movements which may be involved in coupling nucleotide hydrolysis to substrate transport. In our laboratory, we purified the ATPBD of a Cu^{+} transporting P-ATPase from the hyperthermophile archaea *Archaeoglobus fulgidus*. We characterized the effect of substrate concentration and temperature on its steady state ATPase activity observing it could be described by a Michaelis-Menten equation and obtaining K_m and V_{max} as a function of temperature. Additionally, we examined the effect of temperature on its intrinsic fluorescence observing a conformational transition occurring within the temperature range where this protein is catalytically active. A simple 3 states steady-state kinetic model explains the activity dependence on substrate concentration, its optimal working temperature and intrinsic fluorescence changes. Furthermore, since we hypothesized that there is an open-close transition involved in catalysis, we studied the role of local unfolding (“cracking”) on ATPBD activity and found that low urea concentrations induce an increase in ATPase activity and shift the optimal working temperature to lower values. Altogether these results suggest that, in this case, optimal working temperature is, at least partially, modulated by a conformational change.

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A quantitative study of the effect of Ionic strength on the kinetics of an archaeal halophilic enzyme using steady-state and pre-steady-state kinetic approaches. Gabriel Vallejos-Baccelliere[1] (gvallejos@ug.uchile.cl) & Sergio B. Kaufman[2]. [1]Laboratorio de Bioquímica y Biología Molecular, Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Chile. [2]Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires – IQUIFIB (UBA-CONICET), Argentina

The effect of ionic strength on enzyme catalysis has not been quantitatively well studied, with the main insights coming from enzymes with positively charged surfaces interacting with negatively charged substrates (e.g., ribonucleases). In this ongoing work, we studied the effect of salt concentration on the kinetics of a Glucose-6PDH belonging to the halophilic archaeal organism *Haloferax volcanii* (HvG6PDH), which has a negatively charged surface and a negatively charged substrate, glucose-6P (G6P), along with a second substrate with both positive and negative moieties, NAD^+. The enzyme can also use glucose as substrate. Using steady-state kinetic studies, we found that the main effect of salt was on the K_m for G6P, with a decrease of approximately 50-fold at high salt concentrations (2 M KCl). No relevant changes were observed on V_m, while an approximately 5-fold increment was observed for the K_m for NAD^+. For glucose, an approximately 10-fold increase in V_m/K_m was observed, in contrast to a 30-fold increase for V_m/K_m when G6P was the substrate. These results suggest an ionic strength effect on the apparent affinity for G6P due to charge screening by salt concentration, with a concomitant decrease in repulsion between the enzyme and the substrate. We also performed pre-steady-state single-turnover experiments on G6P and glucose oxidation using NAD^+ as the limiting reagent. Interestingly, while the observed rate constant for G6P oxidation did not vary with the salt concentration, it increased approximately 3-fold for glucose oxidation, suggesting a diverse effect of ionic strength on different stages of the catalytic cycle.

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New combination of cisplatin and maraviroc sensitizes drug-resistant gastric cancer cells and improves the survival rate in mice. Bárbara Mora-Lagos[1] (barbara.mora@uautonoma.cl), María Elena Reyes[1], Lorena Lobos-González [2,3], Matías del Campo [2,3], Carmen Gloria Ili[4], Kurt Buchegger[5], Ismael Riquelme[1], Priscilla Brebi[4]. [1]Instituto de Ciencias Biomédicas, Facultad de Ciencias de la Salud, Universidad Autónoma de Chile. [2]Centro de Medicina Regenerativa, Facultad de Medicina-Clínica Alemana, Universidad del Desarrollo. [3]Advanced Center for Chronic Diseases, ACCDiS. [4]Millennium Institute on Immunology and Immunotherapy. Laboratory of Integrative Biology, CEMT-BIOREN, UFRO. [5]Department of Basic Sciences, UFRO.

Gastric cancer (GC) is an important cause of death worldwide. Cisplatin (CDDP) is the most used drug in the chemotherapy of advanced GC. However, resistance to CDDP reduces GC survival. CCR5 has been associated with GC development, but its role in CDDP resistance in GC has not been elucidated. The aim of this study was to determine the effects of CCR5 blockade by a highly selective antagonist (Maraviroc), using AGS R-CDDP cells, tumorids (tumor spheroids) and animal models. AGS R-CDDP cells (CDDP-resistant human gastric adenocarcinoma cells) were previously established using a stepwise drug dosing protocol and characterized. CCL5, the main ligand for CCR5, was selected through transcriptomic analysis and its expression level was validated by qRT-PCR. Cytotoxicity assays were determined by using MTT. Apoptosis and cell cycle assays were evaluated by flow cytometry. Tumoroid formation was performed in low adhesion plates (Nunclon™Sphera™). Animal studies were conducted on immune-compromised BalbC NOD/SCID mice. Maraviroc (MVC) was used alone and in combination with CDDP in all assays. MVC/CDDP combination triggered a re-sensitized phenotype in AGS R-CDDP cells (decreasing cell viability but not increasing apoptosis). AGS R-CDDP cells treated with MVC/CDDP showed: a dose-dependent relationship of CDDP in cell cycle progression; a decrease in CCL5 mRNA levels; and a decrease in the number of tumorids. Finally, MVC improved the survival rate of CDDP-treated mice. MVC/CDDP combination could be used as a potential coadjuvant in GC therapy allowing CDDP doses to be reduced and thus side effects to be reduced.

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Palmitic acid reduces mitochondrial fitness by modulating AMPK activation at the hypothalamic primary cilium. Daniel Peña-Oyarzún[1,2] (daniel.penoy@uv.cl), Catalina Kretschmar[1], María Paz Hernández[1,3], María Chiara Maiuri[4,5,6], Valentina Parra[7,8], Félix A. Urra[9], Eugenia Morselli[3], Alfredo Criollo[1]. [1]Institute for the Investigation in Odontology Sciences (ICOD), Faculty of Odontology, Universidad de Chile, Santiago, Chile. [2]Interdisciplinary Center for Research in Territorial Health of the Aconcagua Valley (CIISTe Aconca-gua), School of Medicine, Faculty of Medicine, San Felipe Campus, Universidad de Valparaíso, San Felipe, Chile. [3]Department of Basic Sciences, Faculty of Medicine and Science, Universidad San Sebastián, Santiago, Chile. [4]Centre de Recherche des Cordeliers, Équipe Labellisée Par la Ligue Contre le Cancer, Inserm U1138, Université Paris Cité, Sorbonne Université, Institut Universitaire de France Paris, France. [5]Metabolomics and Cell Biology Platforms, UMS AMICCa, Gustave Roussy Villejuif, France. [6]Department of Molecular Medicine and Medical Biotechnologies, University of Napoli Federico II, Napoli, Italy. [7]Department of Biochemistry and Molecular Biology, Faculty of Chemical and Pharmaceutical Sciences, Universidad de Chile, Santiago, Chile. [8]Advanced Center for Chronic Diseases (ACCDiS), Faculty of Chemical and Pharmaceutical Sciences, Universidad de Chile, Santiago, Chile. [9]Laboratory of Metabolic Plasticity and Bioenergetics, Program of Molecular and Clinical Pharmacology, Institute of Biomedical Sciences, Faculty of Medicine, Universidad de Chile, Santiago, Chile.

The Chilean population is the second most obese, mainly due to saturated fatty acid consumption. Among these, palmitic acid (PA) stands out given that it is accumulated in the hypothalamus, leading to anorexi-genic neurons dysfunction. Mitochondrial fitness, the homeostatic balance between mitochondrial shape, clearance and function, is diminished after treatment with PA. However, is unknown if mitochondrial fitness is altered in hypothalamic neurons exposed to PA, and if the primary cilium, an antennae-like or-ganelle, is regulating this process. In addition, studies have shown that the AMP-dependent kinase (AMPK) is enriched at the primary cilium. Since AMPK activation is implicated in the upregulation of the mitochondrial fitness, we studied whether PA inhibits AMPK activation at the primary cilium of hy-pothalamic anorexigenic neurons, thus reducing mitochondrial fitness. We treated N43/5 cells, an in vitro model of mouse hypothalamic anorexigenic neurons, with 100µM PA. Mitochondrial fitness was evaluated by assessing mitochondrial fractionation, mitophagy and oxygen consumption rate. We ob-served that PA increased mitochondrial fragmentation, and diminished mitophagy and oxygen consump-tion rate, suggesting that PA decreases mitochondrial fitness. We also demonstrated that PA requires the primary cilium to impair mitochondrial fitness, as disassembly of cilium prevented the effects of PA. Fi-nally, we observed that PA downregulates AMPK activation at the cilium, and that accumulation of AMPK at the cilium, mediated by the downregulation of IFT27 protein, prevents the effects of PA over mito-chondrial fitness. Future studies will address whether ciliary AMPK activation is associated with a fast-ing response.

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Altered Expression of Cardiac Transcription Factors and Differentiation Markers during Cardiomyocyte Differentiation of iPSCs from Down Syndrome Individuals.

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Down syndrome (DS) is a genetic condition that leads to various disturbances in cellular processes, including cardiogenesis, increasing the risk of developing pathologies, such as congenital heart diseases (CHD). Molecular analyses have identified CHD-related genes on chromosome 21 as well as crucial genes for appropriate cardiogenesis. Moreover, transcription factors (TFs), signaling pathways, and the non-coding transcriptome (ncRNA) participate in this complex process. Of note, during cardiac differentiation of DS-induced pluripotent stem cells (iPSCs), cardiac TFs expression were reduced. In our research, we aimed to identify dysregulated early TFs and ncRNAs during DS-iPSC differentiation into cardiomyocytes. IPSCs were differentiated to cardiomyocytes and RT-qPCR was used to evaluate cardiac TFs and long ncRNAs on Days 1, 3, 5, 10, and 15. Dysregulated TFs were found during day 3 (*Factor T, MESP*) and day 5 (*ISL-1*, and *MEF2C*) corresponding to mesoderm and progenitor specification of DS-iPSCs differentiation, respectively; in conjunction with a slowly decline of pluripotency markers (*NANOG*, *POU5F*, and *SOX2*). Also, cardiac differentiation markers (*MYH6*, *GATA4*, *NKX2-5*, and *TNNT2*) were downregulated in DS-iPSCs differentiation compared to disomic iPSCs. Moreover, the lncRNAs *MIAT, LINC00205* and *LINC00698* were upregulated between days 3-5 of DS-iPSCs differentiation, suggesting their implication during cardiac mesoderm specification. In conclusion, the dysregulation in early TFs during cardiac differentiation of DS-iPSCs, affects crucial stages for adequate cardiogenesis, and might be related with the altered expression of lncRNAs involved in this process. Our future work will focus on exploring the relationship between dysregulated TFs and ncRNA during this perturbed process in DS-iPSCs.

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Phlorotannin-containing extracts from Chilean brown seaweeds and curcumin inhibit glycolysis and reduce the metastatic potential of cancer cells. Layla Simón[1,2] (lsimon@uft.cl), Gonzalo Rivera Andrades[1,3], Migdalia Arazo-Rusindo[1,4], Simón Guerrero[5], Felipe Oyarzún-Ampuero[5,6], María Salomé Mariotti-Celis[1,#], Andrew F.G. Quest[2,6,#]. [1] Nutrition and Dietetic School, Universidad Finis Terrae, Santiago 7501015, Chile. [2] Cellular Communication Laboratory, Center for Studies on Exercise, Metabolism and Cancer (CEMC), Institute of Biomedical Sciences (ICBM), Faculty of Medicine, University of Chile, Santiago 8380492, Chile. [3] Institute of Nutrition and Food Technology (INTA), University of Chile, Santiago 7830490, Chile. [4] Department of Chemical and Bioprocess Engineering, Faculty of Engineering, Pontificia Universidad Católica de Chile, Santiago 7820436, Chile. [5] Departamento de Ciencias y Tecnología Farmacéuticas, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago 8380494, Chile. [6] Advanced Center for Chronic Diseases (ACCDiS), Faculty of Medicine, University of Chile, Santiago 8380492, Chile.

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Metastasis is the leading cause of cancer related deaths. Caveolin-1 is a tumor promoter that induces metabolic switching, and thereby enhances the metastatic potential of cancer cells. Brown seaweeds are characterized by their nutritional, antioxidant, and antihyperglycemic properties. Phlorotannins are polyphenols highly present in brown seaweeds. Another natural compound with antioxidant and antitumoral properties is curcumin. Our general aim was to study the effect of phlorotannins obtained from Chilean brown seaweeds (*Durvillae incurvata*) through ultrasound-assisted extraction and curcumin-loaded nanoemulsions on glycolysis and the metastatic potential of Caveolin-1-expressing cancer cells. To that end, brown seaweed extracts and curcumin-loaded nanoemulsions were used to treat metastatic melanoma (B16F10) and breast cancer cells (MDAMB231) over-expressing Caveolin-1 to evaluate their effect on glycolysis, migration and metastasis. Phlorotannin-containing extracts and curcumin-loaded nanoemulsions reduced glycolysis in cancer cells by preventing Hexokinase II activity, and limited thereby the metastatic potential of cancer cells. With these experiments, we determined that extracts obtained from an unexploited Chilean raw material, as well as curcumin-loaded nanoemulsions, present pharmaceutic potential to be used in the future to prevent metastasis and thus reduce cancer-related deaths.

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Targeting gallbladder cancer aggressiveness through recombinant NEP-mediated ET1 degradation. Jetzabel Vidal (jetzabel.vidal@alumnos.uach.cl), David Brown, Verónica Sánchez Hinojosa, Claudia Quezada, Ignacio Niechi. Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile

Gallbladder cancer (GBC) is among the most prevalent malignancies affecting the biliary tract and is associated with high incidence and mortality rates in Chile. In the early stages, there is a lack of clearly defined symptoms, which complicates therapy with conventional approaches. Thus, investigating novel targets capable of mitigating factors promoting tumor development, malignant progression, and aggressiveness holds paramount importance. One such signaling pathway linked to these characteristics is the Endothelin-1 (ET1) pathway, which signals through its G-coupled receptors and is irreversibly degraded by the metalloprotease Neprilysin (NEP). This study aimed to assess whether the application of exogenous recombinant NEP (rNEP) could decrease the ET1-dependent aggressiveness of GBC cells. To achieve this objective, we employed the NOZ cell line and the primary culture CAVE1, subjecting them to rNEP treatment. Protein and transcript levels were measured through western blot and RT-qPCR, respectively. ELISA was employed to quantify extracellular ET1 levels, while transwell and matrigel-coated transwell assays were used to assess cell migration and invasion, respectively. Additionally, tumorigenic capacity was evaluated in vitro using colony formation assays. Results demonstrated that rNEP effectively reduced extracellular ET1 levels and downregulated markers associated with ET1 pathway activation and aggressive characteristics. Furthermore, rNEP treatment led to decreased migration, invasion, and colony formation. In conclusion, our results suggest that the application of rNEP, through ET1 degradation, disrupts key elements involved in tumor progression in gallbladder cancer cells. This study provides new opportunities for potential therapeutic strategies aimed at mitigating the aggressiveness of GBC via targeting the ET1 pathway with recombinant NEP.

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Potential anti-inflammatory effect of endothelial small extracellular vesicles. Ursula Zuniga-Cuevas[1], Leslye Venegas-Zamora[1], Andrés Ramírez-Reyes[1], Ximena Calle[1], Mayarling Francisca Troncoso[1], Valentina Parra[1], Mario Chiong[1], Sergio Lavandero[1], Jaime A. Riquelme[1](riquelme@ciq.uchile.cl).[1]Advanced Center for Chronic Diseases (ACCDiS), Facultad de Ciencias Químicas y Farmacéuticas & Facultad de Medicina, Universidad de Chile, Santiago, Chile.

Endothelial cells produce small extracellular vesicles (sEVs) that contribute to physiological and pathophysiological processes. Whether these sEVs wield anti-inflammatory effects or if this can be exogenously modulated remains unknown. We isolated sEVs from Human umbilical cord vein endothelial cells (HUVEC) treated with or without Interleukin-10 (IL-10), using size exclusion chromatography. To determine size and concentration of sEVs, we used Nanoparticle Tracking Analysis. Expression of sEVs markers was evaluated using immunofluorescence and ELISA assays. Visualization was achieved using Electronic Microscopy. The effect of sEVs was tested in two *in vitro* models: 1) HUVECs undergoing replicative senescence, whereby expression of pro-inflammatory markers Interleukin-6 (IL-6) and p65 was evaluated by qRT-PCR. The senescent phenotype was assessed by β-Galactosidase staining, as well as area and perimeter measurement using fluorescence microscopy. Finally, p16 and p21 were determined by Western blot. 2) Vascular Smooth Muscle Cells (VSCM) A7r5, treated with TNF-α (10 ng/mL) where migration was assessed through wound healing assay. The results show that IL-10 does not affect the size, concentration, morphology, or expression of surface markers of sEVs. Furthermore, 10^8 particles/mL of sEVs from HUVECs treated with or without IL-10 had no effect on senescence-induced expression of IL-6 or p65. However, both types of sEVs reduced VSMC migration elicited by TNF-α. Thus, sEVs derived from endothelial cells treated with or without IL-10 do not reverse the expression of pro-inflammatory markers in senescent HUVECs but do reduce migration of VSMC induced by TNF-α, thus pitching these sEVs as potential therapeutic agents in cardiovascular inflammatory contexts.

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1. Identification of miRNA-155-5p contained in extracellular vesicles from liquid biopsies to predict Glioblastoma Stem Like-Cell subtype. Brenda Águila-Díaz[1] (brenda.aguila@alumnos.uach.cl), Pamela Silva-Álvarez[1], Rodrigo Maldonado-Águila[2], Giovanna Navarro-Martínez[1], Arnaldo Rosales[1], Henry Cabrera[3], Rómulo Melo[3], Claudia Quezada-Monrás[1,4]. [1]Laboratorio de Biología Tumoral, Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile. [2]Laboratorio de Epigenética y RNAs, Instituto de Anatomía, Histología y Patología, Facultad de Medicina, Universidad Austral de Chile, Valdivia, Chile. [3]Instituto de Neurocirugía Asenjo (INCA), Santiago, Chile. [4]Millennium Institute on Immunology and Immunotherapy, Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile

Background. Glioblastoma (GB) treatment failure is mainly caused by a subpopulation called "Glioblastoma Stem-Like-Cells" (GSCs). This subpopulation consists of two subtypes, known as the Mesenchymal (GSC-Mes) and Proneural (GSC-PN), subtypes. GSC-Mes are associated with a more aggressive phenotype, making the identification of GSC-Mes biomarkers highly relevant. Liquid biopsies are one of the minimally invasive strategies to detect biomarkers, in particular, extracellular vesicles (EVs). VEs contain different macromolecules inside, such as microRNAs (miRs). Specifically, miR-155-5p is enriched in EVs from GSCs associated with therapeutic resistance but it has not been characterized in GSC subtypes. Thus, our goal was to identify miR-155-5p levels in EVs from GB patients' plasma to detect the GSC-Mes subtype. **Methodology.** miR-155-5p levels in EVs from GSC subtype cell cultures were measured by RT-qPCR . EVs from cell culture were isolated by ultracentrifugation, meanwhile EVs from the plasma of GB patients were isolated by precipitation with polyethylene glycol. The obtained results were compared with the characterization of the cultures by qPCR and flow cytometry. **Results.** We found increased levels of miR-155-5p in plasmatic EVs and in primary cultures of GB patients that showed markers associated with GSCs-Mes during their characterization. **Conclusions.** Our results suggest that miR-155-5p is a potential biomarker to identify GSCs-Mes from the plasma of GB patients, making it a good technique to detect the most aggressive subtypes in a less invasive manner.

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2. Characterization of NAC transcription factors differentially expressed in *Pinus.*

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NAC transcription factors are a diverse gene family involved in the regulation of diverse biological processes within plants. These proteins present a highly conserved domain, whose function has been associated with processes linked to plant biomass enhancement and stress. Conifers are an important group of gymnosperms that dominate large ecosystems and are of great economic importance in the forest industry. Transcription factors are involved in growth and stress response. In this sense, NAC-type transcription factors would be involved in this response and given the limited information available, the objective of this study is to characterize NAC-type transcription factors in pine that show differential expression in RNAseq libraries. Contigs with differential expression and the characteristic NAM domain were identified. A phylogenetic analysis with 121 sequences from different species was performed, showing 7 clades with NAC sequences involved in different aspects of growth, development and stress response. In Pinus radiata, 6 sequences were grouped in clades related to secondary cell wall formation (clades C and G). The PpNAC11 sequence clustered in clade E, and associated with drought, salinity and ABA. qRT-PCR was performed for the genes PpNAC11 and PrNAC33, observing differential expression of the NAC sequence in embryos and stress. Subcellular co-localization assay shows that PpNAC11 is localized in the nucleus. The results indicate that pine NAC genes may be involved in the stress response.

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3. Optimization of the efficiency of Dye Sensitized Solar Cells (DSSC) by pigments from antarctic and patagonic bacteria. Isabel Alarcón-Fica[1] (isalarcon2016@udec.cl), José Martinez-Oyanedel[1], Karina Crisóstomo[2], Claudia Pérez[2] , Paulraj Manidural[3], Bayron Cerda[3], Sade White Thompson[3]. [1]Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción. [2]Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas Universidad de Concepción. [3]Departamento de Física, facultad de Ciencias Físicas y Matemáticas, Universidad de Concepción.

In the search for renewable energies with low cost and environmental impact, pigment-sensitized solar cells (DSSC) have been developed. These, like conventional crystalline silicon or gallium arsenide solar cells, transform light energy into electricity. DSSCs are de of a photo-electrode capable of capturing light energy through the pigment that is attached to a TiO2, which acts as a semiconductor, it also has a Pt counter-electrode and an electrolyte to close the circuit. The most commonly used pigments for cell sensitization are organometallic complex pigments and ruthenium complexes, all of them with contamination problems as they are toxic and scarce on earth. As a more ecological strategy, natural pigments have been used, the most common being flavonoids, anthocyanins and beta-carotene, these can be extracted from flowers, algae, bacteria, among others. These types of pigments are easily accessible, have a low cost of production and extraction. In this project, pigments were obtained from cultures of 9 bacteria from Patagonia and the Chilean Antarctic, which were extracted with methanol and characterized by UV-Visible and fluorescence spectrum. The extracts were characterized by HPLC to see the pigment content. DSSC-type photovoltaic cells were assembled with the pigments obtained and their efficiency was studied. The pigment obtained from the UdeC-A9 strain presented a higher efficiency than all the literature reports for pigments produced by other bacteria.

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4. Control parameters for long-range spatial correlations in coupled and bistable gene networks. Alejandro Aravena[1,2] (adaravena@uc.cl), Tim Rudge[3], Fernan Federici[1,2]. [1] ANID – Millennium Science Initiative Program, Millennium Institute for Integrative Biology (iBio), Santiago, Chile. [2] Institute for Biological and Medical Engineering, Schools of Engineering, Medicine and Biological Sciences, Pontificia Universidad Católica de Chile. [3] Interdisciplinary Computing and Complex Biosystems, School of Computing, Newcastle University, Newcastle, United Kingdom.

The spontaneous emergence of order from the interaction of individual components is a phenomenon that pervades physical, chemical, and biological systems. *Escherichia coli* cells carrying coupled and bistable synthetic gene networks (SGN) are able to self-organize into long-range spatial coherent patterns and non-trivial scalings in some observables that resemble critical states of a phase transition. We develop a mathematical model that describes both the internal dynamics of the SGNs, and the diffusion of coupling signals between cells. We derive an expression describing the probability of transcription for hybrid promoters regulated by transcriptional activators and repressors. This expression is coupled with the dynamics of essential chemical species of the system and the system of ODEs is numerically integrated. Afterwards, this system is simulated in a two-dimensional lattice to include spatial diffusion effects, and the results are analyzed with the aim of identifying control parameters of phase transitions in coupled and bistable SGNs. Preliminary results indicate that our system is hypersensitive to the concentration of the external inducers, and that there is a crucial balance between the spatial diffusion and the membrane permeability of the inducers. These preliminary results aid us in the search for biological control parameters, which could be tuned to induce collective critical behavior, at the intersection of ordered or disordered phases. This work sheds light on the study of scaling laws for gene networks and provides tools for the engineering of self-organizing gene patterns in artificial cell groups.

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5. Validation of highly negative heat capacities of activation at elevated temperatures in the ADP-dependent sugar kinase family through unfolding kinetics analysis. Ignacio Aravena-Valenzuela (ignacio.aravena.v@ug.uchile.cl), Pablo Maturana, Felipe González-Ordenes, Gabriel Vallejos-Baccelliere, Víctor Castro-Fernández, Victoria Guixé. Laboratorio de Bioquímica y Biología Molecular, Departamento de Biología, Facultad de Ciencias, Universidad de Chile

Life has evolved to prosper in niches with a wide range of temperatures, so, during evolution enzymes from different organisms had to be adapted by modifying their stability and activity. Enzyme activity typically increases with temperature, reaches a peak, and then declines, a phenomenon often ascribed to denaturation. However, the macromolecular rate theory (MMRT) posits that changes in the activation heat capacity ($\Delta C_P^{\ddagger}$) can drive the temperature dependence of enzyme reaction rates, implying temperature-dependent variations of thermodynamic activation parameters ($\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$). In this work, we perform a comparative study on enzymes belonging to the ADP-dependent kinase family. Using steady-state kinetics, we obtained the k_{cat} as a function of temperature and fit the MMRT equation to this data. A highly negative $\Delta C_P^{\ddagger}$ regime at elevated temperatures was found, ranging from -30 to -50 $kJ \cdot mol^{-1} \cdot K^{-1}$.
To validate our approach, we corroborate that the decline in enzyme activity was not due to denaturation by assessing the thermal stability and unfolding kinetics of the enzymes using circular dichroism measurements. We found that the melting temperatures were within the assayed temperature range, suggesting a contribution of denaturation on the decline of activity. However, the unfolding kinetic constants were consistently at least three orders of magnitude lower than the catalytic constants, indicating that the activity measurements are independent of denaturation in the whole range of assessed temperatures. Remarkably, no $\Delta C_P^{\ddagger}$ values of this magnitude have been reported previously, highlighting the need for further studies to elucidate the significance of this parameter in enzyme thermoadaptation.

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6. The antagonism of the adenosine receptor A_{2B} interferes with TGF-β/SMAD signaling in podocytes. Ignacio Arias (iaact.cl@gmail.com), Pablo Mendoza, Claudio Cappelli, Andres Insunza, Rody San Martin. Laboratorio Patología Molecular, Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Chile.

Diabetic Nephropathy (DN) is a complication of diabetes which manifests with glomerulopathy, progressive proteinuria and extensive renal fibrosis that leads to loss of renal function and which currently remains incurable. The fibrotic process conducted by the transforming growth factor-β (TGF-β) is mainly responsible for the loss of kidney function and end-stage renal disease through the triggering of a pathogenic cascade involving epigenetic alterations which includes microRNAs and histone modifications. Recently, it has been shown that the antagonism of adenosine receptor A_{2B} (A_{2B}AR) is able to reduce fibrosis in diabetic rats, thus our aim was to investigate its possible interference on TGF-β activity at the cellular level. Human immortalized podocytes were exposed to TGF-β (10 ng/ml) and the selective A_{2B}AR antagonist MRS1754 (50 nM). The levels of phospho-SMAD2/3 were determined by western blot. The abundance of SMAD2/3 and H3K9 acetylation (H3K9ac) on fibronectin and collagen type I promoters were evaluated through chromatin immunoprecipitation (ChIP) and qPCR. We found that the MRS1754 treatment reduced SMAD3 phosphorylation following exposure to TGF-β in podocytes. Furthermore, the antagonism of A_{2B}AR also led to a reduction of SMAD2/3 binding and H3K9ac abundance on TGF-β target gene promoters. We conclude that the antifibrotic effects of the antagonism of A_{2B}AR involves interference with the profibrotic TGF-β/SMAD signaling cascade in podocytes. This study supports the utility of A_{2B}AR antagonism as a novel tool for the treatment of DN.

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7. Development of prototype multivalent vaccine against Bovine Viral Diarrhea.

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Pathologies caused by the bovine viral diarrhea virus (BVDv) generate huge economic losses in the livestock industry worldwide. The prevention of bovine viral diarrhea is mainly carried out through vaccination campaigns together with the implementation of biosecurity procedures. Conventional vaccines are currently used in BVDv viral spread control. However, the protection reached is insufficient. In this research, a multivalent vaccine prototype was developed to protect animals against infections of viral strains circulating in Chile. For this purpose, the extracellular fragment of the E2 protein corresponding to 5 subgenotypes of BVDv was expressed. E2 proteins were obtained in mammalian cell cultures and purified by metal-ion affinity chromatography. The vaccine prototype was formulated by an equimolar mixture of each E2 antigen in an oil-in-water emulsion. The immunogenicity and safety of the vaccine prototype were evaluated in a sheep model before the immunization assay of cattle. The immune response observed in the animals immunized with the recombinant vaccine was similar to that obtained with the commercial Cattle master vaccine. Also, the cellular immune response regarding changes in the relative expression of IFN-γ and IL-12 showed a significant increase at 56 days post-immunization. The results suggest that the vaccine candidate can generate a similar immune response to the commercial vaccine.

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8. Role of the SALL2 Transcription Factor in Epithelial-Mesenchymal Transition and its Implication in Tumor Malignancy in Colorectal Cancer. Diego Benítez Riquelme[1] (diegobenitez@udec.cl), Manuel Mastel[2], Monserrat Cabrera Aros[1], Aracelly Quiroz[1] (aracellyquiroz@udec.cl), Ariel F. Castro[1], Rene-Filip Jackstadt[2], Iván González Chavarría[1], Roxana Pincheira[1]. [1]Facultad de Ciencias Biológicas, Universidad de Concepción, Chile. [2]Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, Alemania.

Colorectal cancer (CRC) is the second leading cause of global cancer-related deaths. Its primary mortality arises from metastasis, particularly in the advanced stages of the disease, which is when it is commonly diagnosed. An essential process inducing metastasis is the epithelial-mesenchymal transition (EMT), a dynamic phenomenon where epithelial cells acquire mesenchymal traits. Phenotypic traits include increased cellular migration and invasion, higher proliferation, cytoskeletal reorganization, and loss of cellular polarity. At the molecular level, transcription factors linked to EMT (such as Snail, Slug, Zeb1, among others) are responsible for the expression status of crucial genes that uphold epithelial integrity. Within this framework, it is imperative to identify novel factors governing tumor progression and the EMT. Here, we investigated the involvement of the SALL2, a transcription factor significantly downregulated during CRC progression. To this aim, we generated CRC cell lines and organoid models, with gain and loss of SALL2 function, and evaluated phenotypic traits (proliferation, migration, invasion, cytoskeletal reorganization) and molecular changes. We found that loss of SALL2 leads to increased EMT-induced characteristics and the upregulation of mesenchymal markers such as Snail, Slug, Twist1, Zeb1, and N-cadherin. Ours findings underscore the significant impact of SALL2 loss on CRC progression by positively regulating the EMT. Future studies will evaluate *in vivo* how Sall2 loss impacts CRC progression and metastasis.
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9. Exploring Cellular Interactions: P2X4 and 5-HT3A Receptor Crosstalk. Graciela Bravo[1,2] (gsbravo@uc.cl), Xander Sarabia[1], Yuan Chang – Halabi[1], Daniela Villalobos[1], José Cordero[1], Xavier Figueroa[1], Nelson P. Barrera[1]. [1]Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, Chile. [2]Department of Chemical Engineering and Bioprocesses, School of Engineering, Pontificia Universidad Católica de Chile, Santiago, Chile.

Cellular communication through membrane proteins is a prevalent strategy employed by diverse cell types to adapt to complex environments. This study focuses on the interplay between P2X4 and 5-HT3A receptors. These receptors play crucial roles in various pathophysiological contexts, including pain and post-operative nausea and vomiting. Although the mechanisms of their interaction and subsequent cellular repercussions remain elusive, recent investigations have shed light on the impact of stoichiometry and ligand binding on the structure of inhibitory crosstalking in P2X4/5-HT3A receptor complexes. In this context, we propose that the interaction between P2X4 and 5-HT3A receptors at the cell surface leads to a cascade of interconnected intracellular pathways. This phenomenon is governed by ATP binding to the P2X4 receptor and modulated by serotonin binding to the 5-HT3A receptor. To explore this hypothesis, we undertook several experimental steps. First, we transfected the TSA201 cell line to co-express both receptors and validated their membrane presence using TIRF microscopy. Subsequently, we assessed the impact of crosstalk between P2X4 and 5-HT3A receptors on intracellular Ca2+ levels. To study the stoichiometry of the interaction between both receptors, they were purified and visualized by atomic force microscopy. Overall, drawing on these experimental findings, we formulated a putative signaling network using Ingenuity Pathway Analysis. This endeavor contributes to unraveling the intricate landscape of membrane receptor interactions and their downstream cellular consequences.

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10. Inhibiting ECE1 in gallbladder cancer cells: impact on migration and invasion.

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Gallbladder cancer (GBC) is the most prevalent and aggressive cancer in the biliary tract, displaying high incidence and mortality rates in Chile. Endothelin-1 (ET1), a mitogenic peptide, plays a crucial role in cell invasion across several tumor models, with a short half-life of one minute, making its effects entirely reliant on continuous production by endothelin-converting enzyme 1 (ECE1). While the ECE1/ET1 axis has been proposed as a potential therapeutic target, its specific role in GBC remains unexplored. The objective of this study was to investigate the effects of inhibiting ECE1 with SM19712 on migration and invasion in GBC cells. The CAVE1 and NOZ cell lines were employed. Protein levels were assessed using western blot, while extracellular ET1 levels were quantified through ELISA. Cell migration and invasion were evaluated using transwell and matrigel-coated transwell assays, respectively. NOZ cells exhibited higher basal levels of ET1 than CAVE1, but both GBC cell lines displayed comparable ECE1 levels. Inhibition of ECE1 with SM19712 successfully reduced extracellular ET1 levels and consequently decreased cell migration. This was accompanied by a negative regulation of mesenchymal markers and a re-expression of epithelial markers. Interestingly, cell invasion in NOZ cells was slightly decreased under ECE1 inhibition, indicating a potential independent regulatory role of ET1 production. In conclusion, SM19712 effectively decreases the bioavailability of extracellular ET1, mitigates cell migration, but does not entirely reverse cell invasion in this GBC model. These findings shed light on potential therapeutic strategies targeting the ECE1/ET1 axis for managing gallbladder cancer progression.

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11. Evaluation of the molecular and biological differences between Intestinal and Diffuse gastric cancer-derived cell lines. Catalina Jimenez[1,2]*, Andrea Parra[1,2]*, Constanza Cárcamo[1] (constanza.carcamo@falp.org), Troy Ejsmentewicz[1], Karina Cereceda[1], Roxana González-Stegmaier[1], and Franz Villaroel-Espindola[1]. [1]Translational Medicine Laboratory, Instituto Oncológico Fundación Arturo López Pérez, Santiago, Chile. [2]Medical Technology School, Universidad San Sebastián. Santiago, Chile.

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Gastric cancer (GC) is a multifactorial disease, related to *Helicobacter pylori (H. pylori)* infection, high consumption of alcohol and salt, smoking. Based on structural cellular components there are three major GC subtypes: well differentiated (non-cardia/intestinal), poorly differentiated (cardia/diffuse) and mixed disease. Intestinal-GC is predominately found in elder men and better prognoses than other subtypes. The Diffuse-subtype has poor survival and is commonly found in younger women. The mixed-subtype represents a very small group, usually men with highly invasive and metastatic tumor. The aim of this work was to characterize the expression of key biomarkers in NCI-N87 (Intestinal) and KATO-III (Diffuse) cell lines, and its ability to express IL-8 under pro-inflammatory conditions. The basal expression of PD-L1, VISTA, HER2, and Toll-like receptors (TLR4 & TLR5) was evaluated by conventional PCR. NCI-N87 cells qualitatively displayed all mRNAs compared to KATO-III, which only expressed HER2 and VISTA. Later, NCI-N87 and KATO-III cells were cultured in presence of conditioned supernatants derived from peripheral blood mononuclear cells (PBMCs) stimulated with *H. pylori*. The results showed that only KATO-III cells responded and significantly increased the levels of endogenous IL-8 mRNA with a peak at 24H (p-value<0.001). In addition, the stimulation with exogenous IL-8 (20ng/mL) showed a positive feedback on KATO-III cells, which upregulated endogenously IL8 after 48H. NCI-N87 cells were insensitive to any studied condition. Overall, our results confirmed significant molecular and biological differences between GC subtypes, suggesting that the IL-8 response could be associated to the poor outcome in patients with diffuse GC.

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12. Exploring the Regulatory Role of lncRNAs in Chondrogenesis: Towards Targeted Mesenchymal Stem Cell Phenotype Modulation. Verónica Castañeda[1,3,4] (vlcastaneda@miuandes.cl), Gisselle Poblete[3], Gino Nardocci[2,3,4]. [1]PhD Program in Biomedicine, Faculty of Medicine, Universidad de los Andes, Santiago, Chile, [2]Faculty of Medicine, Universidad de los Andes, Santiago, Chile, [3]Molecular Biology and Bioinformatics Lab, Program in Molecular Biology and Bioinformatics, Center for Biomedical Research and Innovation (CIIB), Universidad de los Andes, Santiago, Chile, [4]IMPACT, Center of Interventional Medicine for Precision and Advanced Cellular Therapy, Santiago, Chile.

Long non-coding RNAs (lncRNAs) have recently emerged as pivotal regulators in various cellular processes, with particular relevance to chondrogenesis—an essential mechanism governing cartilage development and maintenance. This study aims to deepen our comprehension of chondrogenesis in Mesenchymal Stem Cells (MSCs) and direct their differentiation toward specific chondrocyte phenotypes. Our methodology involves identifying potential lncRNA candidates through the analysis of gene expression data from a publicly available dataset obtained from a chondrogenesis experiment in bone marrow MSCs (BM-MSCs). Subsequently, we validate the expression of these candidates during chondrogenesis in umbilical cord (UC) MSCs and chondrocyte cultures. Our findings indicate that lncRNAs play a key role in chondrogenesis across diverse MSC sources. Specifically, DANCR, ODIR, and SNHG5 exhibit the expected expression patterns across different types of MSCs during chondrogenesis. Furthermore, we have uncovered a distinct set of lncRNAs associated with a specific fibrocartilage-like phenotype, which we have termed F-lncRNAs. Notably, the expression of F-lncRNAs has also been identified in our fibrocartilage control samples. In light of our results, we conclude that lncRNAs may exert a significant influence on chondrogenesis and possess the potential to be manipulated to direct the differentiation process towards specific chondrocyte phenotypes. We envision that the modulation of lncRNA expression has the potential to enhance the efficacy of MSC therapy by regulating their specific differentiation. This study contributes to our understanding of the molecular mechanisms of lncRNAs underlying chondrogenesis and offers a promising avenue for refining regenerative strategies in the context of cartilage-related disorders.

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13. Unraveling the Interaction Interface of P2X4 and 5-HT3A Homoreceptors: A Step towards Understanding Inhibitory Crosstalk.

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Direct physical interaction between purinergic 2 (P2X) and Cys-loop receptors has been reported. Cells expressing both receptors, when co-stimulated with adenosine-5'-triphosphate (ATP) and 5-hydroxytryptamine (serotonin), generate inhibitory crosstalk through direct cross-inhibition between the responses of each channel. Furthermore, it has been proposed that both the C-terminus of P2X receptor and the second inner loop (IL-2) of the 5-HT_{3A} receptor play crucial roles in this process; however, the exact site of contact between these proteins remains elusive. The experimental characterization of membrane proteins and their interactions is a challenging, time-consuming, and expensive endeavor. Hence, computational approaches, such as molecular dynamics (MD) simulations, have emerged as valuable tools offering complementary solutions to these experimental challenges. In this study, we employed homology modeling to generate the full structures of the P2X type 4 receptor (P2X4) and the 5-HT_3 type A receptor (5-HT_{3A}), including their respective C-terminus and IL-2 domains. Using these models, we made predictions regarding the physical interaction between these two receptors. Subsequently, we assembled and conducted MD simulations to assess the stability, specificity, and essentials components of this interaction. We anticipate that the results from these simulations will provide valuable insights to guide future experimental research, and enhance our comprehension of the molecular mechanisms underlying inhibitory crosstalk between ionotropic receptors in the context of cell signaling.

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14. NUAK1 promotes cancer cell survival through Akt signaling activation. Viviana Coliboro [1]* (vcoliboro@udec.cl), Mario Palma [1]*, Luis-Espinoza[1], Alejandro Farías[1], José L. Gutiérrez[2] Roxana Pincheira[1], Ariel Castro[1]. [1]Laboratorio de Transducción de Señales y Cáncer. [2]Laboratorio de Regulación Transcripcional. Departamento de Bioquímica y Biología Molecular. Facultad Cs. Biológicas. Universidad de Concepción, Chile.

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NUAK1 is a serine/threonine kinase member of the AMPKα-family, whose high expression is associated with poor prognosis in several cancers. However, NUAK1 regulation and functions in cancer remain poorly characterized. We previously identified that NUAK1 interacts with Akt and directly phosphorylates it at Ser-473. Because the hyperactivation or deregulation of the phosphatidylinositol 3 kinase (PI3K)/Akt pathway is associated with tumor initiation and progression, we investigated whether NUAK1 regulates the Akt signaling. Using pharmacological inhibition and shRNA-dependent silencing, we demonstrated that NUAK1 activates the EGFR- and insulin-dependent Akt regulation of the phosphorylation of its downstream substrates FOXO1/3a and GSK3β but not TSC2. We performed cell fractionation, immunofluorescence, and qPCR studies to investigate further the role of NUAK1 in the Akt/FOXO1/3a axis. The NUAK1/Akt/FOXO1/3a axis reduced p21CIP1 and p27KIP1 expression but induced FoxM1 expression. Additionally, using 2D and 3D cultures, we found that NUAK1 promotes cancer cell survival in an EGF-dependent manner, and its inhibition potentiates the effect of MK-2206, an Akt inhibitor. Thus, our studies demonstrate that NUAK1 regulates cancer-associated Akt signaling via direct phosphorylation and indicate that targeting NUAK1, either alone or combined with Akt inhibitors, may be effective in cancers with hyperactivated Akt signaling.

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15. Metabolomics exploration and *in silico* analysis of the VEGF Signaling Pathway in endothelial cells. José Cordero (jocorderf@gmail.com), Mónica Márquez, Matías Muñoz, Graciela Bravo, Xavier Figueroa, Nelson P. Barrera. Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, Chile.

This work focuses on the characterization of the metabolome of endothelial cells through mass spectrometry and the subsequent computational analysis in the context of the VEGF signaling pathway using the Ingenuity Pathway Analysis (IPA) software. Furthermore, we explore the predictive effects of different Nonsteroidal Inflammatory Drugs (NDSAIDs) on the resulting network. First, a metabolomic analysis of endothelial cells was performed through mass spectrometry. Then, an identifier was assigned to each detected metabolite in order to be uploaded to IPA as an organized dataset. The VEGF pathway available in IPA was expanded with the uploaded metabolomic data using the *Grow* and *Path Explorer* tools in order to obtain an expanded network that includes the relationships between the added metabolites and the molecules featured on the pathway. These results revealed key interactions between the uploaded metabolites and molecules in the pathway, providing insights into regulation of important biological functions such as angiogenesis. Finally, using the *Molecule Activity Predictor* tool we studied the predictive effects of different NSAIDs on the expanded network. This simulation permits us to explore how these drugs may have an effect on components of the network and play a role on the functions regulated by the pathway, which may offer relevant information for the design of therapeutic strategies. In conclusion, this investigation combines an experimental and computational approach to study the metabolomic response of endothelial cells in the context of the VEGF pathway and NSAIDs, providing valuable information on the relationship between those drugs, metabolites and biological functions.

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16. Structural and physicochemical studies of recombinant surface immunogenic protein of *Streptococcus agalactiae*. Joaquín Correa[1] (jcorrea2016@udec.cl), Maximiliano Figueroa[1], Abel Vásquez[2], José Martinez-Oyanedel[1]. [1]Laboratorio de Biofísica Molecular, Departamento de Bioquímica y Biología Molecular, Facultas de Ciencias Biológicas, Universidad de Concepción. [2]Sección de Biotecnología, Departamento de salud Ambiental, Instituto de Salud Pública de Chile.

Streptococcus agalactiae, known as β-hemolytic *Streptococcus* or group B (GBS), is a Gram-positive bacterium, the principal etiologic agent associated to fetal morbidity and mortality. GBS colonizes the gastrointestinal and genitourinary tract. It has a world prevalence of colonization during maternity in a range of 11-35%. The infection with GBS is responsible of 90.000 deaths in minors and 57.000 fetal deaths in the world. The surface immunogenic protein (SIP) is a highly conserved protein in most serotypes of GBS. The subcutaneous, intranasal and oral immunization with recombinant SIP (rSIP) generates specific opsonophagocytic antibodies. Besides, rSIP stimulates cellular and humoral immune response. However, there is no experimental structural information about rSIP. In this project, we present physicochemical and structural studies of rSIP (AVV_1) and a truncated variant (AVV_2). These proteins were obtained from recombinant *E. coli* and purified in IMAC column. Circular dichroism (CD) and thermic denaturation studies were realized in the spectropolarimeter Jasco 1500. Dimerization studies were performed through Disperse Light Scattering (DLS). According to these studies AVV_1 has a Tm of 60,2°C, and presents 14% helix, 22% strand, 18% turns and 46% unfolded structures and it is a dimer. AVV_2 has a Tm of 57,0°C, and presents 14% helix, 20% strand, 18% turns and 48% unfolded and it is a monomer. The secondary structures obtained from the CD are quite different form the ones established from the available Alpha-fold model. These differences allow us to suppose that AVV_1 and AVV_2 has different structured areas and a more compact folding.

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

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

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18. Identification of critical residues for the HMP-phosphate kinase activity in the vitamin kinase family. Cortés-Rubilar Isaac (isaac.cortes@ug.uchile.cl) , Fuentes-Ugarte Nicolás, Pérez Myriam, Herrera Sixto, Vallejos- Baccelliere Gabriel, Guixé Victoria, Castro-Fernández Víctor. Laboratorio de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Chile, Santiago, Chile.

Thiamine biosynthesis in bacteria requires the formation of hydroxymethyl pyrimidine diphosphate (HMP-PP), through two phosphorylation steps on hydroxymethyl pirimidine (HMP), involving formation of hydroxymethyl pyrimidine phosphate (HMP-P) as an intermediate. These consecutive reactions are carried out by the *thiD* gene product, an enzyme with HMP-phosphate kinase activity (ThiD-HMPPK). The second reaction of phosphorylation corresponds to a methyl phosphate kinase activity ($-CH_2-PO_3$), a unique activity in the vitamin kinase family, whose mechanism is unknown. To address the determinants of the HMP-phosphate kinase activity, the ligand-complexed crystal structure of an ancestral ThiD-HMPPK from *Enterobacteriales* (AncEnHMPPK) was determined. From this, two residues seem to be involved only in the second phosphorylation of HMP: His179 and Thr211, stabilizing the ATP molecule. The role of these residues (His179 and Thr211) in the AncEnHMPPK activity was assessed by site-directed mutagenesis. We constructed single and double mutants (H179A, T211A, and H179A/T211A), which were kinetically characterized. Single mutants, H179A and T211A, show slightly differences in kinetic parameters with HMP as substrate, whereas for HMP-P, a decrease in $k_{cat,}$ of 5-fold and 14-fold were observed for H179A and T211A, respectively. On the other hand, the H179A/T211A double mutant shows no activity with HMP-P (second phosphorylation) and only a slight decrease in k_{cat} with HMP as substrate. These results suggest that both residues, His179 and Thr211, are critical for the second phosphorylation of HMP, but not for the first one. Substrate interactions of these residues have been evaluated by Molecular Dynamics simulations.

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19. Identification of Candidate Loci in the Molecular Characterization of Acute Myeloid Leukemia Driven by MLL-AF9 Fusion Protein. Diego Cuevas[1] (dcuevas@magister.ucsc.cl), Adolfo Agurto[1] Roberto Amigo[1], Matias Hepp[1], Valentina González-Pecchi[1], Pía Vidal[1], Carolina Benitez[2] and Carlos Farkas[1]. [1]Laboratorio de Ciencias Biomédicas, Universidad Católica de la Santísima Concepción, Chile. [2]CREAV Bío-Bío, Chile.

Acute Myeloid Leukemia (AML), primary based on hematopoietic stem cell dysfunction and transformation, is one of the most aggressive leukemia forms, especially prevalent among adults. It's largely characterized by driver mutations, with the fusion gene t(9;11) (MLL-AF9) taking center stage in this research. Given the low survival rate—only 30-35% in young adults and further diminishing to 10-15% for those over sixty—there's a pressing need for deeper understanding and novel interventions. In this work, we successfully transplanted CRISPR Cas9 GFP-tagged MLL-AF9 positive AML cells into unirradiated Wild Type C57BL6 mice. Remarkably, these mice did not show signs of graft-vs-host disease over a span of three months post-transplantation. Our histological analysis revealed an extensive invasion of AML cells across critical organs including the spleen, liver, and even the brain. Cell citometry readings further validated the presence of GFP in the peripheral blood over time. This non-ablative transplantation method offers a novel experimental approach in AML. Next Generation Sequencing data further confirms the presence of MLL-AF9 gene across Bulk RNA-seq. Notably, a strong expression of MLL-AF9 fusion transcript was detected in more tan 80% of the profiled cells. These experimental approach offers a new model to study in vivo AML, with the opportunity to knock out in vivo genes in secondary AML context via CRISPR-Cas9 technology.

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20. Understanding the role of Neuraminidase I in the polarization of macrophages in a fibrotic context. Emilia Escalona (emilia.escalona@uautonoma.cl), Andres Herrada, Alexandra Olate, Sofia Albornoz and Noelia Escobedo. Lymphatic Vasculature and Inflammation Research Laboratory, Facultad de Ciencias de la Salud, Instituto de Ciencias Biomédicas, Universidad Autónoma de Chile, Talca, Chile.

Macrophages are immune cells that adapt to environmental stimuli through macrophage polarization. They adopt M1-like or M2-like phenotypes for inflammation or tissue repair, respectively. Imbalance in macrophage polarization impacts on many pathologies, but the underlying mechanisms remains unclear. Fibrosis is characterized by connective tissue accumulation and heightened TGF-β, an immunomodulatory cytokine. Fibrotic secondary lymphoid organs (SLOs) have been reported to show a weak immune response to vaccines, but the impacts on immune cell differentiation remain unexplored.
We used Neuraminidase 1 knockout ($Neu1^{-/-}$) mice, a systemic fibrosis model, to understand the implications of fibrotic SLOs in macrophages polarization. Because Neu1 is a sialidase involves in immune regulations, we also tested it role on macrophage polarization.
First, we evaluated *in vitro* polarization capacity of Neu1-deficient macrophages to M1 or M2 stimuli and evaluating by flow cytometry M1/M2 markers. Macrophages showed normal polarization but elevated M2-marker (CD206) expression, linking this receptor to Neu1.
In contrast, $Neu1^{-/-}$ mice displayed fewer M1-like SLOs macrophages, fewer M2-like lymph node (LN) macrophages, and more M2-like splenic macrophages. Additionally, we found high expression of TGF-β in LN from $Neu1^{-/-}$ mice, suggesting a role in downregulating M2-like macrophages. To approach that, we stimulate with TGF-β macrophages *in vitro*, observing a decrease in M2-markers like LN in $Neu1^{-/-}$ mice.
In conclusion, Neu1-deficient macrophages had increased expression of CD206 receptor, which could be contributing to make them less inflammatory. TGF-β affects macrophage polarization, bearing implications for fibrotic diseases. Modulation of NEU1 and/or TGF-β signaling could allow to shape M1/M2 inflammatory responses.

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21. NUAK1 kinase is a novel regulator of Akt activation. Luis Espinoza[1] * (luespinoza2018@udec.cl), Mario Palma[1]*, Alejandro Farías[1], Viviana Colíboro[1], José-Leonardo Gutierrez[2], Roxana Pincheira[1], Ariel Castro[1]. [1]Signal Transduction and Cancer Laboratory, Department of Biochemistry and Molecular Biology, Faculty of Biological Sciences, University of Concepción, Concepción, Chile. [2]Transcriptional Regulation Laboratory. Department of Biochemistry and Molecular Biology. Faculty of Biological Sciences. University of Concepción, Chile.

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The phosphatidylinositol 3 kinase (PI3K)/Akt pathway is essential in tumor initiation and progression. The activation of Akt involves its phosphorylation in two residues: the threonine-308 (Thr-308) and the serine-473 (Ser-473). The Ser-473 phosphorylation is crucial for the Akt activation that leads to downstream regulation of critical cellular processes involved in tumorigenesis, such as cell survival, proliferation, growth, and metabolism. Interestingly, analysis of human tissue data (TGCA) revealed that NUAK1 expression positively correlates with EGFR expression and Akt Ser-473 phosphorylation in human cancers. NUAK1 (ARK5) is a Ser/Thr kinase member of the AMPK-related family, composed of 12 kinases related by sequence homology with the catalytic domain of the AMPK-α subunit. Collectively, these kinases regulate cell adhesion, polarity, metabolism, and the response to different stresses, including energetic, osmotic, and oxidative stress. This study identified that NUAK1 interacts with Akt and induces Akt Ser-473 phosphorylation upon growth factor stimulation. We first demonstrated that NUAK1 interacts with and directly phosphorylates Akt at Ser-473 by in-vitro assays. By a Proximity Ligation Assay (PLA), we confirmed the interaction between these proteins upon EGF stimulation of MDA- MB-231 breast cancer cells. Additionally, we found that NUAK1 is involved in the early EGF-dependent Akt ser-473 phosphorylation and activation in different cancer cells. Our results demonstrate that NUAK1 is a novel direct regulator of Akt ser-473 phosphorylation and activation, suggesting that NUAK1 is involved in the activation of Akt signaling in cancer.

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22. Establishing a technique for the *in vivo* modeling of colorectal tumors in mice.

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Colorectal cancer (CRC) is a worldwide health burden with high incidence and mortality. Despite the increasing understanding of CRC biology and numerous therapeutic advancements in recent decades, preclinical in vivo models are still crucial for identifying and confirming novel biomarkers and therapeutic targets. Most genetically engineered mouse models (GEMMs) of CRC are limited by tumor formation in the small intestine, high tumor burden that limits metastasis, and the need to generate and cross mutant mice, which is expensive and time-consuming. In this study, we set up a CRC modeling approach at the University of Concepción involving colonoscopy-guided injection of sgRNA viral particles in the colon of ROSA26-Cas9-EGFP mice. We performed colonoscopy-guided mucosal injection of lentiviruses containing a sgRNA targeting the *Apc* gene, the initiating mutation in ~80% of human CRC. After the injection, tumor formation was monitored via colonoscopy every 2-3 weeks. Immunohistochemical and histological analysis confirmed the expected tumor changes generated for the *Apc* deletion. We were able to set up a novel CRC model, which offers several advantages over traditional GEMMs: 1) techniques require short training; 2) allows rapid assessment of gene function in tumorigenesis; 3) allows tumor induction and interrogation of any gene in mice without germline mutations; 4) like in the human, tumors form in the distal colon; 5) tumors can be monitored with colonoscopy. In future studies, we will interrogate *in vivo* whether loss of novel cancer-associated genes impacts CRC initiation and progression and CRC therapy.

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23. Evaluation of the potential PGPRs isolated from *Asparagus officinalis* on growth and yield of *Zea mays L*.

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Microbial biotechnology employes techniques that rely based on the natural interactions that occur in ecosystems. Bacteria, including rhizobacteria, play an important role in plant growth, providing agricultural crops with an alternative that can mitigate the negative effects of abiotic stress, such as those caused by saline environments and the increase in the excessive used of the chemical fertilizers. In this study, bacterial isolates were obtained from soil and roots of *Asparagus officinalis* cultivar UF-157 F2 in Virú, Trujillo from the department of Lambayeque, Peru. This region has high salinity levels; therefore, the collected samples were used to isolate plant growth-promoting rhizobacteria (PGPR), which were identified through morphological, and physical-biochemical characteristics. These salt tolerant bacteria were screened phosphate solubilization, indole acetic acid, deaminase activity and molecular characterization by 16S rDNA sequencing. Fifteen samples from saline soils of the *Asparagus officinalis* plants in the northern coastal desert of San Jose district, Lambayeque, Peru. The bacterial isolates were screened for salt tolerance ranging from 3 to 6%, a total of 96 isolates were found. Isolates 05, 08, 09 and 11 showed maximum salt tolerance, quantification of ammonium, phosphate solubilization and IAA production. The four isolates were identified by sequencing the amplified 16S rRNA gene and were found to be *Enterobacter* sp. 05 (OQ885483), *Enterobacter* sp. 08 (OQ885484), *Pseudomonas* sp. 09 (OR398704) and *Klebsiella* sp. 11 (OR398705). These microorganisms promoted the germination of *Zea mays L*. plants and increased the germination rates for treatments with chemical fertilizers in 100% and 50% and PGPRs obtained increased the height and root length of 40 days after planting. The beneficial effects of salt tolerant PGPR isolates isolated from saline environments can be new species, used to overcome the detrimental effects of salt stress on plants. The biochemical response and inoculation of the three isolates prove the potential of using these strains as a source of products that can be employed for the development of new compounds proving their potential as biofertilizers for saline environments.

24. Characterization of the reverse transcriptase of the LTR- retrotransposon Steamer. Maira Fuentes Muñoz[1] (m.fuentesmuoz2@uandresbello.edu), Javiera Avilés[2], Cesar A. Ramirez-Sarmiento[2,3], Luis Brieva de Castro[4], Gloria Arriagada Inostroza[1]. [1]Instituto de Ciencias Biomédicas Universidad Andrés Bello, Santiago, Chile. [2]Institute for Biological and Medical Engineering, Schools of Engineering Medicine and Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, Chile. [3]ANID – Millennium Science Initiative Program – Millenium Institute for Integrative Biology (iBio), Santiago, Chile. [4]National Laboratory of Genomics for Biodiversity, Center for Research and Advanced Studies, Langebio-Cinvestav Sede Irapuato, Guanajuato, México.

LTR-retrotransposons are class I transposable element characterized by the presence of long terminal repeats (LTRs) directly flanking an internal coding region. As retrotransposons, they mobilize through reverse transcription (RT) of their mRNA and the further integration of the newly created cDNA into another location. We have previously described Steamer, an LTR-retrotransposon of the soft-shell clam *Mya arenaria*, highly expressed in leukemic clams. Steamer encodes a polyprotein of 1336 amino acids. Between amino acids 434-854 a conserved domain for RT is predicted. Here we aimed to characterize the RT activity of the putative RT domain of Steamer protein.
We cloned Steamer-RT to express it and purify it from *E coli* in fusion to His-SUMO. The protein was soluble when expressed in *E. coli* BL21 Rosetta (DE3), and induced with 1 mM IPTG for 16 hours at 16°C. The RT activity was measured using the homopolymer assay and an intercalant fluorescent agent with either Mg^{2+} or Mn^{2+} as the preferent cation. This assay was standardize using recombinant RT of MLV. Under the tested conditions Steamer RT has lower activity than MLV RT. Steamer activity was evaluated at 10, 20 and 30°C. At 10°C a higher RT activity was detected, which was greater when Mn^{2+} was used. So far, we have used His-SUMO-RT protein, we are currently testing if the remotion of SUMO will allow an increase in the activity of this enzyme.

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25. Sexual Dimorphism in Mitochondrial Dynamics in a Doxorubicin- Induced Cardiac Senescence model. Wileidy Gómez[1,2] (wileidygomez20@gmail.com), Ingrid Oyarzun[1,2], Georthan Mancilla[1,2], Clara Quiroga [1,2], Pablo Castro[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.

Cardiovascular diseases (CVDs) continue to be a leading global cause of death. Epidemiological evidence has shown that CVDs incidence is lower in premenopausal women compared to men, but significantly higher in postmenopausal women. This reveals a protective effect of female sexual hormones, particularly estrogen, on the cardiovascular system. Estrogen indirectly affects mitochondria by binding to their nuclear receptors, altering the expression of transcription factors such as NRF1 and PGC1α, crucial to electron transport chain complexes transcription, mitochondrial biogenesis and production of reactive oxygen species. The mitochondrial dysfunction arising from oxidative stress during aging contributes to CVD development. For this reason, maintaining of homeostasis mitochondrial through the mechanisms of mitochondrial dynamics is crucial to preserve cardiac function. Using a transcriptomic analysis, we identify differentially expressed genes (DEGs) of mitochondrial dynamics in heart tissue from young and old male and female rats, and hope validated at the DEGs mRNA and protein expression level, in cardiomyocytes derived from neonatal rats (female and male) subjected to doxorubicin-induced senescence, and supplemented with 17β estradiol (E2). We found Mtfr1 gene downregulated in females and upregulated in males; while Mtfp1 showed upregulated in both aged males and females. Addionally, Mic60 and Mic19 showed upregulated only in males, becoming them in interesting targets to validation. In cardiomyocytes treated with E2, the immunofluorescence analysis revealed that the cell damage marker yH2AX decreased in comparison to cells treated with doxorubicin. We expect that our results allow us to propose possible therapeutic targets, and establish diagnostic criteria for CVD in men and women.

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26. Targeting p53-E6 interaction for novel HPV treatment strategies through synthetic peptides. Rayén González (cgonzalezr@udec.cl), Maximiliano Figueroa. Laboratorio de Biofísica Molecular, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.

Cervical uterine cancer is the second leading cause of death for women of reproductive age worldwide, with Human Papillomavirus (HPV) infection accounting for over 90% of the cases. Currently, there is no treatment for HPV infection, and while vaccines exist against the riskiest serotypes (HPV-16, HPV- 11, HPV-18, and HPV-6), their effectiveness is linked to the age of vaccinated women, and leaving out other carcinogenic serotypes such as HPV-30 and HPV-31.

The HPV oncoprotein E6 interacts with p53, polyubiquitinating and degrading it via proteosome, leaving basal cells of the uterine cervix epithelium apoptosis-deficient. Preventing the sequestration and degradation of p53 is vital to avoid the proliferation of infected cells. Therefore, synthetic peptides are proposed to disrupt the interaction between the p53-E6 complex. These peptides could have therapeutic potential against HPV infection, including serotypes that are not targeted by current vaccines.

To accomplish this goal, 30 synthetic peptides in total were generated using three distinct bioinformatics methodologies: based on the structure of p53, *in silico* improvement of this peptide, and peptides design utilizing Artificial Intelligence. Subsequently, their stabilities were evaluated through Molecular Dynamics simulations (MD). They underwent blind and directed Docking against E6 to assess their interaction with the oncoprotein. Positive complexes, indicating those in which the interaction is anticipated at the interface where p53 typically interacts with E6, were selected, and their stability was further examined through MD. Peptides demonstrating high stability within the protein complex, and those predicted to have a low Kd, are suggested as candidates for testing in *in vitro* assays.

27. FrankPEPstein: A novel in silico approach to design therapeutic peptides against target proteins. Joaquin Gutiérrez Benavente[1] (jmgutierrez4@uc.cl), Daniel Garrido[2], Andreas Schüller[1,3]. [1]Department of Molecular Genetics and Microbiology, School of Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, Chile. [2]Department of Chemical and Bioprocess Engineering, School of Engineering, Pontificia Universidad Católica de Chile, Santiago, Chile. [3]Institute for Biological and Medical Engineering, School of Engineering, Medicine and Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, Chile.

Advancements in the past ten years have propelled the evolution of peptide pharmaceutical development, attributed to emerging technologies in production, mutation, and analysis. Peptides are intrinsically capable of exploring larger surface receptors and triggering intracellular effects with high affinity and specificity, thus bearing a high therapeutic value in treating illnesses including cancer, infectious and cardiovascular diseases. In this work, we aimed to construct a new unsupervised and open-source approach for peptide design given a target protein pocket. For this purpose, we have developed an algorithm that analyzes interactions of peptide-protein complexes retrieved from structural databases and then extrapolates this information to binding pockets of proteins of interest. Our method evaluates the contact surface area of each complex and then generates peptide k-mers, storing them with their respective protein residues environment, named "mini-pockets." Each mini-pocket is then structurally aligned with target pockets, placing its respective peptide k-mer onto the target pocket surface. Finally, all the k-mers positioned in the target pockets, obtained from different experimentally validated complexes, are clustered and combined to obtain new peptides of larger size. We validated FrankPEPstein by reconstructing peptides from 200 different complexes of known peptide-protein interaction. We compared each predicted peptide with the crystalized one for every complex through several parameters obtaining promising results. As a projection, we will generate a library of candidate peptides that could inhibit the bacterial enzyme CutC (present in intestinal microbiota) by binding to certain regions critical for enzyme bioactivity, with the potential to aid in preventing cardiovascular disease complications.

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28. Characterization of small extracellular vesicles from breast cancer patients and healthy donors.

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Extracellular vesicles (EVs) play a key role in promoting tumor hallmarks through cell-to-cell communication. Additionally, they hold promising applications in therapeutics and molecular diagnostics. Breast Cancer (BC) ranks as the most diagnosed cancer worldwide, creating a demand for improved and less invasive diagnostic and monitoring tests. In this study, our objective was to characterize and evaluate differences in the size, concentration, and content of small extracellular vesicles (psEVs) derived from the blood plasma of BC patients and healthy donors. To investigate this, sEVs were isolated from the plasma using size-exclusion chromatography. Subsequently, we analyzed psEVs using Nanoparticle Tracking Analysis to determine their diameter and concentration. Furthermore, we characterized psEVs by assessing their total protein content and examining the presence of sEV markers through Western Blotting and Flow Cytometry. Our findings revealed a significant increase in psEV concentration in BC patients compared to healthy donors; however, their diameters did not exhibit significant differences. EV markers were identified in both populations. Nevertheless, patients with BC exhibited higher concentrations of total proteins in psEVs. These results underscore the potential of psEVs as biomarkers. Further exploration into their specific biomolecular content could lead to the discovery of improved biomarkers for the diagnosis and monitoring of breast cancer.

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29. Glycogen Phosphorylase from methanogenic archaea: a highly regulated enzyme.

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Methanogenic archaea have a crucial function in the carbon cycle through their biological production of methane, known as methanogenesis. Although these organisms are unable to assimilate sugars or other complex organic substrates, they synthesize glycogen, which can be degraded under nutrient-depleted conditions. In this metabolic switch, the enzyme glycogen phosphorylase (GP) plays a crucial role. In eukaryotic organisms, GP enzymes are pivotal regulatory points for sugar metabolism, being highly regulated by diverse allosteric effectors, like activated by AMP and inhibited by ATP and glucose-6-phosphate. Despite the key role of GP in the glycogenolysis of methanogenic archaea, no kinetic characterization or allosteric regulation has been reported for archaeal enzymes.

We characterized the GP from *Methanococcus maripaludis* (MmGP) that was recombinantly expressed in *E. coli*. The enzyme is highly specific for glycogen, being this activity approximately 30-fold higher compared to other sugar polymers. The kinetic parameters showed 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 eukaryotic enzymes. Analysis of potential effectors of the MmGP enzyme shows that the enzyme is highly regulated being inhibited by NaPPi, F6P, PEP, ADP, ADP-glucose, and UDP-glucose and activated by fructose-1,6-BisP (FBP). The IC_{50} values for most of the inhibitors are in the micromolar range (50–150 μM), as the AC_{50} for the activator FBP. These results identified new effectors for the archaeal enzyme, contributing to our understanding of methanogenic metabolism and its potential biotechnological applications.

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30. *Helicobacter pylori* activates the HIF-1α/ALS2/Rab5 signaling pathway to induce β-catenin nuclear translocation in gastric cells. Daniela Herrera[1,2,3,5] (dfhr23@gmail.com), Álvaro Neira[3,4], Lucas Faundes[2,3,5], Manuel Varas[3,4], Andrew F.G. Quest[1,3], Vicente Torres[2,3,5]. [1]Laboratory of Cellular Communication, Faculty of Medicine, Universidad de Chile [2]Laboratory of Cellular Biology, Faculty of Dentistry, Universidad de Chile [3]Advanced Center for Chronic Diseases (ACCDiS), [4]Universidad San Sebastián, [5]Millennium Institute on Immunology and Immunotherapy.

Introduction: *Helicobacter pylori (H. pylori)* is a bacterium that has been classified as a class I carcinogen. We previously reported that *H. pylori* induces HIF-1α stabilization. On the other hand, HIF-1α promotes expression of the Rab-GEF ALS2, leading to activation of the small GTPase Rab5 in different tumor cell models. Furthermore, Rab5 activation is involved in endosomal sequestration of the β-catenin destruction complex (DC), which increases nuclear β-catenin levels. However, whether *H. pylori* activates Rab5 and promotes β-catenin nuclear translocation and their dependency on ALS2 and HIF-1α, remain unknown.
Methodology: MKN-74 and AGS gastric cancer cells were infected with *H. pylori*-26695 and Rab5 activation was determined in Rab5-GTP pulldown assays. The dependence of HIF-1α and ALS2 on Rab5 activation was measured in the presence of the HIF-1α inhibitor SC-205346 and using ALS2 *knockdown* cells. Moreover, HIF-1α, ALS2 and Rab5 protein levels were assessed post-infection with *H. pylori* by Western blotting. The endosomal sequestration of DC was evaluated using proteinase-K degradation assays and indirect immunofluorescence. Finally, the nuclear β-catenin levels were determined by indirect immunofluorescence.
Results: Rab5 activation post-*H. pylori* infection was observed. Furthermore, Rab5 activation was determined to be dependent on the stabilization of HIF-1α and ALS2. Additionally, *H. pylori* increased HIF-1α and ALS2 protein levels. Finally, *H. pylori* infection promoted endosomal sequestration of DC and nuclear translocation of β-catenin.
Relevance/projections: This study uncovers an unexpected signaling pathway induced by *H. pylori* and identifies HIF-1α, ALS2, Rab5 and β-catenin as novel potential therapeutical targets in gastric cancer treatment.

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31. Identification and characterization of YAB transcription factors and anthocyanin accumulation under UV-B stress conditions in cultivated tomato (*Solanum lycopersicum* 'Indigo Rose'). Karla Jara-Cornejo[1,2,3] (karla.jara@utalca.cl), Paz E. Zúñiga[2,3], Claudia Rivera-Mora[2,3], Carlos R. Figueroa[2,3] & Simón Ruiz-Lara[1,3]. [1]Functional Genomics Laboratory, Institute of Biological Sciences, Talca Campus, Universidad de Talca, Talca, Chile. [2]Laboratory of Plant Molecular Physiology, Institute of Biological Sciences, Talca Campus, Universidad de Talca, Talca, Chile. [3]Millenium Nucleus for the Development of Super Adaptable Plants (MN-SAP), Santiago, Chile.

The cultivated tomato (*Solanum lycopersicum*) is one of the most important horticultural crops in the world due to its great nutritional and commercial value. This value is due to the presence of vitamins, carotenoids, and phenolic compounds. Today, different tomato cultivars vary in size, shape, and color of the fruits. The cultivar 'Indigo Rose', unlike most other cultivars of cultivated tomatoes, has a purple coloration which is related to a greater accumulation of anthocyanins in the fruit peel. Anthocyanins correspond to secondary metabolites that are responsible for the coloration of different plant organs, but also participate in multiple processes, including protection against abiotic stress. Anthocyanin biosynthesis is regulated by the interaction of different transcription factors. Among them, the YABs (a.k.a., YABBY), which have been shown in recent studies, are also involved in plant tolerance to abiotic stress. In this research, we study at a physiological level the accumulation of anthocyanins in the fruits of the 'Indigo Rose' tomato. We observed that a purple color appears on the fruit peel from the early stages of development, which correlates with the increasing accumulation of anthocyanins. In turn, we identified and characterized the YAB gene family in the *Solanum lycopersicum* genome. We analyzed their genomic position, gene structure and characteristic domains. In addition, we observed its gene expression profile in different tissues by RT-qPCR, both under normal conditions and under UV-B stress conditions. The results obtained here can help to decipher the role of these new transcription factors in the regulation of anthocyanin biosynthesis under abiotic stress conditions in crop species, such as tomato.

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32. Changes in DNA methylation control transcriptome reprogramming during sulfate deficiency in *Solanum lycopersicum.*

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Sulfur (S) is an essential macronutrient for plants, as a constituent of relevant macromolecules. Currently, the deficiency of Sulfate, the main source of S in soils for plants, has become widespread in soils due to changes in agricultural practices and strict controls over industrial S emissions. Since Sulfate limitation has detrimental effects over plant growth and yield, it is of paramount importance to determine the molecular mechanisms that plants utilize to sense and respond to changes in sulfate availability. We previously characterized the transcriptomic response of tomato to sulfate deficiency, finding a myriad differentially expressed genes, part of which might be mediated by changes in DNA methylation. To determine the impact of DNA methylation on gene response to sulfate, we conducted whole genome bisulfite-seq analysis on tomato roots and shoots. We found thousands of differentially methylated regions (DMRs) in response to sulfate deficiency, some of which were associated with promoter regions of differentially expressed genes. Some of these genes correspond to sulfate transporters and sulfate metabolic genes, suggesting an epigenomic regulation of expression of S-related genes. Moreover, we used the information on DMRs to scan these regions for recognition motifs for transcription factors (TFs) finding TF candidates that might change their accessibility to promoters depending on sulfate availability. One of these TFs is EIL3, a close homolog to SLIM1, a key regulator of the sulfate response in Arabidopsis. In sum, our work points at a relevant function for epigenome reprogramming on gene expression regulation of key sulfate-related genes.

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33. Functional investigation of histone H3 in mitochondria. Scarleth Larraín Goicovich (scarlethlarrain@gmail.com), 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.

Histones H1, H2A, H2B, H3 and H4 are basic proteins of eukaryotic cells that play an essential role in the structure, arrangement, and assembly of DNA into chromatin. While histones were initially thought to be found only in the nucleus of cells, recent studies have shown that they are also present in other cellular fractions such as mitochondria.
Mitochondria are dynamic organelles with diverse functions, including a link to epigenetic processes. Although histone H3 has been found in mitochondrial fractions, the exact purpose of the interaction between histone H3 protein and mitochondria is not yet fully understood. The aim of this work is to determine the presence and function of histone H3 in the mitochondria of different cell lines. On this basis, we investigated the presence of histone H3 in mitochondrial fractions of human cancer cells and mouse embryonic stem cells. We also examined how inhibition of protein synthesis and induction of cell apoptosis affect the presence of histone H3 in mitochondria. All results were analyzed and compared by Western Blot analysis. The results suggest that histone H3 is present in mitochondria and plays a non-canonical, i.e. unconventional, role.

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34. Copper-mediated epigenetic regulation of the commensal pathogen *Enterococcus faecalis.*

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Transcriptional regulation is commonly associated with transcription factors and non-coding RNAs, but another important layer is DNA methylation, where DNA bases are chemically modified to modulate transcriptional machinery affinity. Recent studies have highlighted the significance of bacterial methylations in various physiological processes, including virulence, motility, and metal homeostasis. For pathogenic species like *Enterococcus faecalis*, efficiently managing metal ion fluctuations, particularly copper, is crucial to prevent toxicity. In this study, we investigated changes in the methylation patterns of *E. faecalis* exposed to a non-toxic concentration of copper (0.5 mM $CuSO_4$, 3 hours). Using third-generation sequencing (SMRT and bisulfite), we identified a total of 918 methylation patterns across the promoters of E. faecalis (55% of the total genome). These methylations included three types reported in bacterial species: 5mC, 4mC, and 6mA. Interestingly, 20% of these methylation patterns changed in response to copper exposure, indicating a significant epigenetic response induced by the metal. Subsequent analysis of transcriptome data (RNA-seq) revealed altered transcriptional outputs for 91 genes, suggesting potential impacts of methylation changes induced by the metal. Most of these genes encode for basal metabolism, membrane synthesis, and transport of primary and secondary metabolites, all of which are general functions not directly involved in homeostasis or copper resistance. This suggests that the induced epigenetic changes are not specific to the direct effects of the metal. Understanding the regulatory role of DNA methylation in response to copper opens a new perspective on global gene regulation induced by metals in bacterial species.

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35. A Diabatic Model of Intermediate Stabilization for *In Silico* Catalytic Assessment of the DNA Polymerase β. Mauricio Sandoval[1] and Ricardo A. Matute[1,2] (rmatute@caltech.edu). [1]Facultad de Ciencias, Universidad Mayor, Santiago, Chile. [1,2] Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California, USA.

The catalytic mechanism of DNA Polymerase B has been studied using theory and computational methods in order to improve the understanding of the catalytic effects which play a significant role when comparing the native enzyme against the D256E and D256A mutants. The mechanism in the enzyme involves three steps, namely: (1) proton transfer, (2) nucleophilic attack on the incoming nucleotide, and (3) leaving group departure. The proton transfer leads to the kinetics dependence on the pH for both native and mutants. After the proton transfer, the catalysis follows a stepwise associative mechanism, although with a strong concerted character due to the presence of a very shallow intermediate. Hence, we have applied the theoretical framework of the Diabatic Model of Intermediate Stabilization (DMIS) to get insights into such a mechanistic feature of the enzyme. The appearance of an intermediate is well described by our model in terms of a hidden intermediate activation. We further assessed our model using the Empirical Valence Bond (EVB) Method as implemented for computer simulations of enzyme catalysis, which incorporates both dynamics and electrostatic effects. We found a plausible path via a hidden pentavalent intermediate when the simulation is calibrated using the concerted pathway in the potential energy surface.

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36. Regulation of SALL2 by the Wnt/β-catenin pathway in colon cancer cells. Paula Medina (pmedina2018@udec.cl), Angela Ortiz, Aracelly Quiroz, Ariel Castro, Roxana Pincheira. Signal Transduction and Cancer Laboratory, Dept. Biochemistry and Molecular Biology, Universidad de Concepción.

SALL2, a member of the SALL family of transcription factors, is associated with organ development and involved in proliferation, migration, and apoptosis. SALL2 mRNA is significantly downregulated in colorectal cancer (CRC), and its protein expression decreases during CRC progression. In the search for possible regulatory mechanisms, we and others identified that the SALL2 promoter has binding sites for TCF/LEF, transcription factors of the Wnt/β-catenin pathway. SALL2 protein sequence analyses identified putative phosphorylation sites for GSK3-β and PARSylation sites for Tankyrase, also associated with the Wnt/β-catenin pathway. Because of the significance of the Wnt/β-catenin pathway in normal development and cancer and considering that SALL2 is a regulator of gene expression, we aimed to determine whether the Wnt pathway regulates SALL2 expression and possible associated mechanism(s). Using activators (L- WRN, CHIR99021) and inhibitors (XAV939) of the Wnt/β-catenin pathway in normal colon epithelial and CRC cell lines, we evaluated changes in SALL2 expression by western blot and RT-qPCR. SALL2 is dynamically regulated upon the Wnt/β-catenin pathway activation, significantly increasing its expression at early treatment times. By luciferase reporter and protein stability assays, we showed that the increase in SALL2 expression involves transcriptional and post-translational mechanisms. On the other hand, inhibition of the Wnt/β-catenin pathway using XAV939 (Tankyrase inhibitor) increased SALL2 expression at later times. Treatment with XAV939 generated an apoptotic response, which appears to be SALL2-dependent. As a projection of the study, we intend to deepen the mechanisms of SALL2 regulation by the Wnt pathway and evaluate how its regulation impacts CRC cells' behavior.

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37. Clade-wide proteome analysis shows widespread non-canonical DCR proteins in Fungi. Lorena I. Melet[1,2,3](lorena.melet@mayor.cl), Ivana Orellana[1,3], J. Andrés Rivas-Pardo[1], Nathan R. Johnson[1,2], Elena A. Vidal[1,2]. [1]Centro de Genómica y Bioinformática, Facultad de Ciencias, Ingeniería y Tecnología, Universidad Mayor, Chile. [2]ANID-Millennium Science Initiative Program-Millennium Institute for Integrative Biology (iBio), Chile. [3]Programa de Doctorado en Genómica Integrativa, Vicerrectoría de Investigación, Universidad Mayor, Chile.

Dicer (DCR) is a class-III ribonuclease with a fundamental role in the synthesis of small RNAs and the regulation of gene expression. DCRs can be found in many eukaryotic organisms, including fungi, where DCRs are shown to be essential for defense against virus, transposon regulation and development. To date, fungal DCRs have mainly been identified in the most studied phyla: ascomycetes, basidiomycetes, and chytridiomycetes. However, there are still many fungal species where these proteins have not yet been described. Moreover, there is not a clear understanding of the structural domains present on fungal DCRs. In recent years, there has been an increase in the availability of fungal genomes and proteomes, with more than 1,500 annotated and reference genomes that have annotated proteomes. In this study, we characterize and identify fungal proteins *in silico* on a large scale in more than 1,400 species, that span 9 phyla of the fungal kingdom. We found that a small fraction of proteins contains all the canonical domains described for plants and animals. Most fungi lack a PAZ domain, crucial for sRNA length, determined by distance from DCR protein's RNAseIII core. Our analysis shows that PAZ domains are only present in DCR proteins of the Mucoromycota and Microsporidia phyla. Interestingly, we found PAZ in fungal species that are known symbionts of plants. Our results suggest that the presence of PAZ in these fungi might create sRNAs of similar length to those produced by plants, serving as communicating molecules during interspecies interactions.

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38. The recombinant D1 domain of flagellin enhances the immune response generated by *Helicobacter pylori* in human immune cells.

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Helicobacter pylori (*H. pylori*) is a relevant risk factor in the development of gastric cancer (GC) due to its ability to create a pro-oncogenic environment through virulence factors and immune evasion mechanisms. *H. pylori* flagellin has amino acids substitution within the binding domain that impairs the immune activation by Toll-like receptor 5 (TLR5) in the host. A recombinant peptide (rND1) derived from *Vibrio anguillarum* flagellin has shown *in vivo* and *in vitro* immune adjuvant properties, both in human and non-human models. The aim of this work was to demonstrate that rND1 enhances the *in vitro* response against *H. pylori* mediated by isolated human peripheral blood mononuclear cells (PBMCs). PBMCs were isolated from healthy donors, cultured and stimulated with *H. pylori* both in the absence and presence of rND1. The relative expression of IL-8 was measured through qPCR and the formation of extracellular traps was evaluated by immunofluorescence. The results showed that in immune cells treated with rND1 alone, the IL-8 mRNA increased by 20-fold; however, in combination with *H. pylori*, it increased up to 46 times compared to the control. Similarly, the release of extracellular traps was observed when using rND1 independently; however, in conjunction with *H. pylori*, it led to the formation of cellular clusters by PBMCs. This work confirmed that rND1 enhances the innate immune response against *H. pylori* inducing the formation of extracellular traps and the expression of IL-8. It suggests a novel approach to reduce the risk of GC related to a persistent *H. pylori* infection.

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39. TPC1-Type Channels in Physcomitrium patens: Interaction between EF-Hands and Ca^{2+}. Franko Mérida-Quesada[1] (franko.meridaq@utalca.cl), Fernando Vergara-Valladares[1], María Eugenia Rubio-Meléndez[2], Naomí Hernández-Rojas[2], Angélica González-González[3,4], Erwan Michard[4], Carlos Navarro-Retamal[4,5] and Ingo Dreyer [2]. [1]Programa de Doctorado en Ciencias mención Modelado de Sistemas Químicos y Biológicos, Universidad de Talca, Talca, Chile. [2]Electrical Signaling in Plants (ESP) Laboratory–Centro de Bioinformática y Simulación Molecular (CBSM), Facultad de Ingeniería, Universidad de Talca, Talca, Chile. [3]Programa de Doctorado en Ciencias mención Biología Vegetal y Biotecnología, Universidad de Talca, Talca, Chile. [4]Instituto de Ciencias Biológicas, Universidad de Talca, Campus Talca, Avenida Lircay, Talca, Chile. [5]Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD, USA.

Two-pore channels (TPCs) are members of the superfamily of ligand-gated and voltage-sensitive ion channels in the membranes of intracellular organelles of eukaryotic cells. The evolution of ordinary plant TPC1 essentially followed a very conservative pattern, with no changes in the characteristic structural footprints of these channels, such as the cytosolic and luminal regions involved in Ca^{2+} sensing. In contrast, the genomes of mosses and liverworts encode also TPC1-like channels with larger variations at these sites (TPC1b channels). In the genome of the model plant Physcomitrium patens we identified nine non-redundant sequences belonging to the TPC1 channel family, two ordinary TPC1-type, and seven TPC1b-type channels. The latter show variations in critical amino acids in their EF-hands essential for Ca^{2+} sensing. To investigate the impact of these differences between TPC1 and TPC1b channels, we generated structural models of the EF-hands of PpTPC1 and PpTPC1b channels. These models were used in molecular dynamics simulations to determine the frequency with which calcium ions were present in a coordination site and also to estimate the average distance of the ions from the center of this site. Our analyses indicate that the EF-hand domains of PpTPC1b-type channels have a lower capacity to coordinate calcium ions compared with those of common TPC1-like channels.

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40. Structural and transcriptional characterization of *pyruvate decarboxylase (PDC)* gene family during strawberry fruit ripening process. Francisca Hormazábal-Abarza[1], Darwin Sáez[1,2,], Francisca Rodríguez-Arriaza[1], Daniel Bustos[3,4], Gabriela Urra[3], Ángela Méndez-Yáñez[1], Patricio Ramos[4,5], Luis Morales-Quintana[1] (luis.morales@uautonoma.cl). [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, 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]Plant Microorganism Interaction Laboratory, Instituto de Ciencias Biológicas, Universidad de Talca, Talca, Chile.

The organoleptic quality of a fruit encompasses various attributes, including texture, shape, sweetness, color, nutritional composition, and aroma. The aroma perception, which contributes significantly to overall fruit quality, is reliant on retronasal olfactory perception by consumers. Additionally, authors have described that at least one gene encoding the PDC enzyme would be involved in the ripening of strawberry fruits (*Fragaria x ananassa*). The objective of this work was to analyze the relative expression changes of the gene family encoding the PDC enzyme, in different ripening stages of strawberry fruits and treated with the plant hormones auxin and ABA. By conducting a search within the *F. x ananassa* genome database, eight *FaPDC* gene sequences were retrieved. Subsequent multiple sequence alignments and phylogenetic analyses identified characteristic domains unique to this gene family, associating them with other PDCs implicated in ripening processes. The transcriptional behavior of this gene family was assessed, revealing a subset of genes that exhibited heightened expression levels throughout the fruit ripening stages. Based on the deduced enzyme sequences, 3D models of the proteins were constructed, followed by the evaluation of their interactions with potential ligands. Lastly, through an analysis of the promoter regions, it was determined that these genes contain *cis-elements* responsive to hormones. This was substantiated by hormone treatments and subsequent transcriptional assessments of the gene family. In conclusion, the *FaPDC* family presents changes in genetic expression and its hormonal regulation during the ripening of strawberry fruits.

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41. DRP1 and Bcl-xL interaction in the chemotherapeutic induced cellular senescence progression of colorectal cancer.

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Colorectal cancer (CRC) is one of the leading causes of death. CRC chemotherapies promote cell death mechanisms, such as apoptosis. However, cells can follow alternative fates to death, like cellular senescence. This state corresponds to a stable cell cycle arrest, accompanied by different key aging features. In various cancers, low levels of the mitochondrial fission protein DRP1 promotes susceptibility to chemotherapeutics. However, there is no consensus regarding the mechanisms underlying this phenomenon, and whether it occurs in CRC. Interestingly, it has been reported that DRP1 interacts with the anti-apoptotic protein Bcl-xL, which is key for senescence induction. Thus, the interaction between both proteins could promote Bcl-xL mitochondrial localization and the establishment of cell senescence instead of death. To explore this, we used the CRC cell lines HCT-116 wild type, DRP1*KO* and MFF*KO*. As a senescence inducer, sublethal doses of Doxorubicin were used. Survival and cell senescence were evaluated by crystal violet staining; flow cytometry viability probes; staining for SA-β-galactosidase activity; caspase activation and the accumulation of P53, P21 and P16, which were evaluated by Western blot. We also evaluated the pharmacological inhibition and silencing of DRP1 and Bcl-xL, as well as the interaction between both proteins by confocal microscopy. The reduction of DRP1 levels, but not its pharmacological inhibition or the absence of MFF (another mitochondrial fission protein), promote cell death during senescence induction. Additionally, a DRP1/Bcl-xL co-compartmentalization was evidenced during senescence induction. To our knowledge this is the first report evaluating the importance of the DRP1-Bcl-xL interaction in CRC.

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42. Structural insights into the regulation of bifunctional fructose-1,6- bisphosphate aldolase/phosphatase from methanogenic archaea. Sebastián M. Muñoz[#] (sebastian.munoz.m@ug.uchile.cl), Felipe González-Ordenes[#], Víctor Castro-Fernández and Victoria Guixé. Laboratorio de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Chile. Santiago, Chile.

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Methanogenic archaea are unable to grow using hexose sugars as their sole carbon source. Nevertheless, these microorganisms can accumulate glycogen, which can be metabolized into intermediates for methanogenesis when nutrients are scarce, but no regulatory mechanism has been described for this gluconeogenic/glycolytic metabolic switch in methanogenic archaea. Recently, our group reported that the glycolytic bifunctional ADP-dependent glucokinase/phosphofructokinase enzyme is allosterically activated by AMP in *Methanococcus maripaludis.* Then, it is interesting to address whether the reverse reaction catalyzed by the bifunctional fructose 1,6-bisphosphate aldolase/phosphatase (MmFBPA/P) is also regulated, constituting a novel regulatory point in *M. maripaludis* metabolism.
The bifunctional FBPA/P catalyzes the aldolic condensation of dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (GAP), and the dephosphorylation of fructose-1,6-bisphosphate (F1,6BP) to fructose-6-phosphate (F6P). The phosphatase reaction is essentially irreversible in gluconeogenesis and is allosterically regulated in most of the organisms studied.
We determined the kinetic parameters for both activities of the recombinant MmFBPA/P and assessed the regulation of the phosphatase activity by several effectors, determining that it is activated by the effector cAMP. To gain information on the structural determinants of this regulation, we employed cryo-EM. The first cryo-EM model for a methanogenic archaeal FBPA/P at 6.39 Å resolution was determined, displaying a homooctamer arrangement formed by two concentric rings around a central pore. Based on this model, a potential allosteric site for this enzyme was proposed, suggesting structural insights into its regulation. The results obtained will contribute to metabolic studies of methanogenic archaea, enabling potential engineering applications such as biomass generation and industrial starch processing.

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43. Tissue-specific codon usage bias in expressed genes of *Drosophila melanogaster*.
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The redundancy of the genetic code implies that different 3-letter combinations of nucleotides can give rise to the same amino acid, which are known as synonymous codons. The frequency at which each synonymous codon is used varies at gene, tissue, and species level. Codon usage bias can be explained by nucleotide composition of the genome (mutational bias) and translational selection. Furthermore, genes expressed in different tissues are subject to different regimes of translational selection and therefore differ in their translation pattern. To study codon usage adaptation in *Drosophila* three tissue specific transcriptomes were analyzed: brain, fat body, and imaginal wing disc. Codon usage bias was evaluated taking into consideration tissue specific gene expression levels for each coding sequence in the genome. When analyzing codon usage globally in each tissue specific transcriptome no obvious bias was observed. However, when the analysis was restricted to the genes with the higher and lower expression in each tissue, the bias in codon usage differed remarkably. In highly expressed genes the codon usage bias is towards optimal codons (most used), in contrast, genes with reduced expression show minimal codon usage bias. These results suggest that the genes expressed at the level of different tissue present distinctive regimes of translational selection and genes expressed in more than one tissue analyzed would display differential translation efficiencies.

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44. EGFR/PI3K/AKT1 downregulation and $A_{2B}AR$ antagonism affect mesenchymal markers in glioblastoma stem-like cells. Giovanna Navarro-Martínez[1] (giovannanavarro@alumnos.uach.cl), Bárbara Hidalgo-Sotomayor[1], Pamela Silva-Álvarez[1], Rubén Yañez[2], Mariela Silva[2], Diego Carrillo[1,3,4], Claudia Quezada-Monrás [1,4].
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Background. Glioblastoma (GB) is a highly aggressive brain cancer. GB's main treatments include surgery, chemotherapy with temozolomide, and radiotherapy. Low treatment effectiveness and treatment resistance are attributed to glioblastoma stem-like cells (GSCs) and specifically to their mesenchymal (MES-GSCs) rather than the proneural (PN-GSC) subtype. High levels of adenosine in the GSCs microenvironment correlates with the activation of low-affinity adenosine receptors, such as $A_{2B}AR$, which promote cell invasion, proliferation, immune suppression, and angiogenesis. Additionally, highly amplified and mutated EGFR in glioblastoma cause similar effects. The activation of these receptors can promote MES-GSC differentiation and could be involved in the over- activation of the PI3K/Akt1 pathway. We aimed to establish a treatment that sensitizes MES-GSC to radiation by regulating mesenchymal-proneural transition.
Methodology. MES-GSCs were treated with an $A_{2B}AR$ antagonist for 24 hours before radiation (10Gy), protein microarray, and flow cytometry assay were performed to evaluate proneural and mesenchymal markers. MES-GSC were treated with $A_{2B}AR$ antagonist and/or EGFR- tyrosine kinase inhibitor for 12h western blot assay was performed to assess PI3K/Akt1 pathway activation.
Results. Antagonism of $A_{2B}AR$ before radiation diminished mesenchymal markers and enhanced proneural markers. Combined treatment, $A_{2B}AR$ antagonist, and EGFR-tyrosine kinase inhibition, showed a summatory effect by downregulating the PI3K/Akt1 pathway even without radiation.
Conclusions. We suggest that a combined effect generated by the antagonism of EGFR and adenosine receptor A_{2B} may influence radiation-induced mesenchymal to proneural transition, via the downregulation of the PI3K/Akt pathway, in glioblastoma stem-like cells. This treatment combination could increase patient survival after radiation.

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45. Development of a cancer cell model for the functional study of NSD3S/MYC protein-protein interaction. Yanara Núñez[1] (ynunez2017@udec.cl), Víctor Baeza[2], Valentina González-Pecchi[2]. [1]Bioquímica, Universidad de Concepción, Chile. [2]Laboratorio de Investigación en Ciencias Biomédicas, Facultad de Medicina, Universidad Católica de la Santísima Concepción, Chile.

MYC is a transcription factor that acts as an oncoprotein, since its overexpression results in tumor progression. Among the proteins that regulate MYC function, is the histone methyltransferase NSD3. Specifically, the short isoform of this protein, NSD3S, is capable of interacting with MYC, increasing its transcriptional activity and promoting its stability, inhibiting the FBXW7-mediated degradation of MYC. Interestingly, deletion of amino acids 389-404 of NSD3S (NSD3S△15), doesn't promote MYC transcriptional activity and stability. Since the NSD3S/MYC interaction could be a new target on the therapy of MYC-driven tumors the main goal of this project is to develop cancer cell models for the study of this interaction, by the generation of stable cells lines with NSD3S wild-type (WT) or NSD3S△15. Lentiviral plasmids of GFP-NSD3SWT and GFP-NSD3S△15 were generated, correct gene insertion was confirmed by sequencing. Stable and transient HT-29 (colon cancer) and HeLa (cervical cancer) cell models were characterized by fluorescence microscopy and expression of the protein of interest by Western blot. As an approximation for the functional assays, the cancer cell models were tested by RT-qPCR to see differences in gene expression, specifically in MYC target genes. The generation of the cell models, NSD3S WT and NSD3S△15, will be a tool used for the functional characterization of the effect of NSD3S/MYC interaction in MYC amplificated cancer cells. The goal of this study is to characterize NSD3S/MYC interaction, as an oncogenic interaction, and as a potential drug interface for MYC-driven tumors.

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46. FluOpti: Low cost and open source optogenetic imaging system for synthetic spatial biology. Isaac Núñez[1,2] (innunez@uc.cl), Tamara Matute[1,2], Daniel Núñez[2,3], Wladimir Araya[4], Domingo Gallardo[4], Fernan Federici [2,3]. [1]Departamento de Ingeniería Química y Bioprocesos, Escuela de Ingeniería, Pontificia Universidad Católica de Chile (PUC). [2]ANID – Millennium Science Initiative Program, Millennium Institute for Integrative Biology (iBio), Santiago, Chile. [3]Institute for Biological and Medical Engineering, Schools of Engineering, Medicine and Biological Sciences, Pontificia Universidad Católica de Chile**.** [4]IOWLabs, Santiago, Chile**.**

The spatial organization of cell states plays a critical role in the functioning of biological systems. Understanding the self-organization of these patterns remains a fundamental challenge in Biology. Classical approaches to understanding multicellular organization are limited by the analytical capacity of natural biological systems. The high number of components, complexity of their interactions, and limited manipulation make their study challenging. We have developed a synthetic biology and open hardware combined platform to study and manipulate the patterning behavior of synthetic gene networks (SGNs). This is composed of 1) modular genetic components, including the CcasS/R optogenetic system and LuxR/LasR quorum sensing system, to elaborate combinatorial synthetic gene networks designs, and 2) an open hardware equipment that allows two-wavelength optogenetic control, green light (520 nm) and red light (660 nm), which are used as an environmental input to trigger SGNs in *E. coli* cells. It also involves blue light (470 nm) and white light illumination to perform multi fluorescence and brightfield imaging, together with ITO glass heating devices to perform temperature control. The design is based on a Raspberry Pi camera and microcontroller making it easy to program to satisfy different regimenes of control and timelapse registry. The equipment proved to be effective in collecting high-quality data on colony growth and gene expression, as well as an efficient optogenetic regulation. As a whole, the developed experimental system constitutes a simple, affordable and versatile platform for studying and programming complex phenomena of cellular organization in a traceable manner.

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47. Environmental DNA (eDNA) detection of *Giardia duodenalis* and *Castor canadensis* trough LAMP reaction and homebrew reagents. Tamara Matute[1,2] , Daniel Núñez [2,3] (dnuez@uc.cl), Isaac Núñez [1,2], Fernan Federici [2,3]. [1]Escuela de Ingeniería, Departamento de Ingeniería Química y Bioprocesos, Pontificia Universidad Católica de Chile. [2] ANID – Millennium Science Initiative Program, Millennium Institute for Integrative Biology (iBio), Santiago, Chile. [3]Institute for Biological and Medical Engineering, Schools of Engineering, Medicine and Biological Sciences, Pontificia Universidad Católica de Chile.

One of the major problems affecting conservation of ecosystems is the presence of invasive alien species (IAS). This factor not only causes a great deterioration of biological diversity and ecosystem damage but also leads to significant economic losses. International recommendations point to early monitoring strategies as the most economical and effective option that allows carrying out early prevention plans for the control of invasive species and zoonotic diseases. However, one of the main barriers to controlling and containing IAS, and its related pathogens, is the lack of adequate monitoring systems and the difficulty of mobilizing resources for their execution. This work presents the development of open access biological resources, open hardware and free software for the monitoring of environmental DNA in aquatic ecosystems, mainly focused on the detection of the *Castor canadensis* and *Giardia duodenalis* in the field. This includes the development of locally produced molecular tools, the preparation and optimization of low-cost LAMP reactions, the selection of specific targets and proper primer design pipelines to improve the performance of reactions with environmental samples. We also describe the development of open hardware equipment for the registration and analysis of fluorescent signals. The results demonstrate the low-cost and decentralized molecular detection of *Castor canadensis* and *Giardia duodenalis* DNA. Furthermore, the performance of these reactions was comparable to commercial kits in terms of sensitivity and specificity. This work contributes to the development of molecular detection techniques in the field, epidemiological surveillance, and/or species conservation.

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48. Science, Policy and Society: the role of biochemists and biochemistry in the Chilean scientific policy institutions between the years 1957 and 1980. Cristóbal Olivares[1,3] (colivaresv@fen.uchile.cl), Christian A.M. Wilson Moya[1], Bárbara Silva Avaria[2]. [1]Single Molecule Biochemistry and Mechanobiology laboratory. Department of Biochemistry and Molecular Biology. Faculty of Chemist and Pharmaceuticals Sciences, Universidad de Chile. [2]Department of History. Faculty of History, Geography and Political Science. Pontificia Universidad Católica de Chile. [3]School of Postgraduate. Faculty of Economy and Business, Universidad de Chile.

In 1957, the University of Chile creates the first experimental science career in the country: Biochemistry. This career sparked new possibilities for scientific labor and public space for professional scientists. Later, in 1967, President Frei created the National Commission of Science and Technology, which was highly influenced by this new generations of professionals. Among these scientists, it can be distinguished for their scientific contributions and participation in the public policy: Osvaldo Cori, cofounder of the Biochemistry career; Hermann Niemeyer, founder of the first Ph.D. program in science; Jorge Allende, coordinator of the first Molecular Biology classes in the country. From these personalities, the relationship between biochemistry and the national science institutionalization can be traced.
This sociohistorical and science policy research aims to analyze and interpret the scientific contributions of these pioneers in biochemistry to the institutionalization and strengthening of the experimental sciences between 1957 and 1980. We discovered connections between these investigators and how the lack of resources and poor conditions of making science pushed them to seek for representativity and lead them to important position in the principal institutions that administrates scientific research. We also discovered that their research directly enhanced the national scientific work and the national scientific institutions, but the growth of their scientific research work was inversely proportional with their public roles and actions. To finalize, I'll briefly talk about the projections of where this kinds of research in the study of contemporary scientific institutions, public policy and science policy area can lead.

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49. A new method to measure ATP-dependent kinase activity: Kinetic characterization of a coupled assay involving ADP-dependent glucokinase and glucose-6-phosphate dehydrogenase enzymes. Martín Pereira-Silva (martin.pereira@ug.uchile.cl), Nicolás Fuentes-Ugarte, Victoria Guixé and Víctor Castro-Fernández. Laboratorio de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Chile, Santiago, Chile.

ATP-dependent kinase activity is usually measured with a pyruvate kinase and lactate dehydrogenase (PK/LDH) coupled assay, where the ADP production is coupled to the oxidation of NADH which is quantifiable by UV-Visible spectrometry at 340 nm. However, some problems related to this assay are the high cost of the enzymes and the instability of PEP employed as a co-substrate. We present a simple coupled assay that uses an ADP-dependent glucokinase from *Thermococcus litoralis* (TlGK) and a glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides* (LmG6PDH) enzymes, coupling the ADP production to NAD^+ reduction. We kinetically characterized both enzymes in the enzyme assay conditions, and determinate their kinetic parameters (KM and $Vmax$) at pH 7.0 and 25°C. Enzyme units (U/mL) required for the coupled assay were calculated using McClure and Storer mathematical models. Subsequently, we test the assay measuring the hydroxymethyl-pyrimidine phosphate kinase (HMPPK) activity. Inhibition of TlGK and LmG6PDH by assay components was evaluated, where ATP behaves as a weak inhibitor of both enzymes. For LmG6PDH ATP shows a competitive inhibition with respect to the nucleotide NAD^+ and a mixed inhibition when glucose-6P was the varied substrate. This analysis allowed us to design a reliable coupled assay effective for a broad range of ATP concentrations (from 0,01 up to 10 mM) for measuring HMPPK activity. These findings enable us to explore other assay conditions, broadening the spectrum of kinase activity that may be determined through this method.

Fondecyt 1221667

50. Role of ET-1 in the stability and co-transcriptional activity of β-catenin in gallbladder cancer cells. Pilar Püschel[1] (puschelpilar@gmail.com), Verónica Sánchez Hinojosa[1], Julio C. Tapia[2], Ignacio Niechi[1]. [1] Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile. [2]Programa de Biología Celular y Molecular, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Santiago, Chile

Gallbladder cancer (GBC) stands as one of the most aggressive malignant neoplasms affecting the biliary tract, arising from abnormal epithelial gallbladder cell growth, which complicates diagnosis and therapy. The endothelin axis (ET) and its receptors (ETAR and ETBR) play a crucial role in vascular homeostasis and tumor progression. One of its isoforms, ET-1 promotes aggressiveness through β-catenin, activating genes that foster pro-tumoral characteristics. The co-transcriptional activity of β-catenin relies on its protein stabilization and translocation to the nucleus. However, whether ET-1 is involved in β-catenin regulation in GBC cells remains unknown. Based on these background findings, we aim to determine whether ET-1 regulates β-catenin function, which could impact GBC tumor progression. For this purpose, we assessed nuclear protein levels and β-catenin transcript levels using western blot and RT-qPCR, respectively, in GBC cells (NOZ) treated with ET-1 and MAC (dual ETRs antagonist). Stability of β-catenin was evaluated by a cycloheximide assay and its localization by immunofluorescence microscopy. Finally, β-catenin co-transcriptional activity was evaluated by TOP/FOP-flash reporter assay. Our results demonstrated that ET-1 stabilizes β-catenin, consequently enhancing its co-transcriptional activity. While ET-1 marginally elevates nuclear β-catenin levels, a notable increase in its total protein levels and stability is observed. This finding suggests that despite not inducing significant changes in its localization, ET-1 effectively heightens β-catenin's co-transcriptional activity in GBC.

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51. Application of Optical Tweezers for Detecting Misfolding Intermediates of RNA Molecules Induced at Low Temperatures. Rodrigo Rivera (rodrigo.rivera.s@ug.uchile.cl), Cristian Valdebenito, Cayetana Zamorano & Mauricio Baez. Laboratorio de Bioquímica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.

Correct folding of RNA is crucial for its proper function. In this context, the temperature is a key variable affecting the interconversion rates between the conformational states of the RNA. Specifically, a temperature downshift decreases the rate of interconversion favoring the presence of misfolding RNA structures. However, bulk experiments performed by classic methodologies like circular dichroism or electrophoretic mobility shift assays lack the required resolution to characterize the misfolding distribution of states induced by a temperature downshift. In this work, we used an optical tweezers device to characterize how the temperature affects the folding pathways and the misfolding probability of a small harping of 53 ribonucleotides. At 10 °C, the force necessary for the unwinding of the RNA hairpin increases in comparison to experiments conducted at 21 °C. Also, the number of mechanical trajectories with cooperative transitions of unfolding and refolding diminishes, which can be explained by the stabilization of folding intermediaries or misfolded structures induced by low temperatures. Ongoing experiments are currently underway to investigate how RNA chaperones, induced by cold conditions, influence the mechanisms underlying RNA folding and misfolding.

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52. Impact of Hormone Treatments on Phenolic Content, Color Development, and Pigment-Related Gene Expression in Strawberries during Pre and Postharvest Phases.

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Globally, strawberry fruits are immensely popular, yet certain environmental and physiological conditions hinder their red color development, causing economic losses due to reduced product quality. To investigate the relationship between color changes and nutritional compounds driven by two important hormones involved in the fruit ripening process, we conducted a comparative study on the "Camarosa" strawberry fruits. We analyzed anthocyanin accumulation, total phenolic and flavonoid content, and examined pigment-related gene transcription profiles post Abscisic acid (ABA) and auxin (Aux) hormonal treatment in pre and postharvest conditions. Thus, following ABA treatment, we observed heightened red coloration, indicative of positive color enhancement in white stage fruit (preharvest conditions), and an increase of the red color in the ripe stage (postharvest condition). However, auxin treatment during the two stages of strawberries did not yield significant color changes. Moreover, anthocyanin accumulation increased substantially post ABA treatment, affecting the transcriptional profile of genes involved in flavonoid pigment biosynthesis. Notably, the *FaANS* gene demonstrated the most pronounced increase. Excitingly, we established correlations between color and vital physiological properties like SSC/TA and weight. This underscores color's role as a quality indicator. The implications extend to future breeding programs, where these findings can inform decisions aimed at enhancing the nutritious compound content of strawberries.

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53. Intracellular processing of mollusk hemocyanins as complex non-specific immunostimulant proteins by antigen-presenting cells. Michelle L. Salazar[1] (michelle.salazar@ug.uchile.cl), Claudia d'Alencon[1], Diego Díaz-Dinamarca[2], Augusto Manubens[3], Fabián Salazar[1,4], María Inés Becker[1,3]. [1]Laboratorio de Inmunología, Fundación Ciencia y Tecnología para el Desarrollo (FUCITED). [2]Sección de Biotecnología, Subdepartamento, Innovación, Desarrollo, Transferencia Tecnológica (I+D+T) y Evaluación de Tecnologías Sanitarias (ETESA). Instituto de Salud Pública. [3]Investigación y Desarrollo, Biosonda S.A. [4]Medical Research Council Centre for Medical Mycology, University of Exeter, Exeter, United Kingdom.
Mollusk hemocyanins are widely used as adjuvants, carriers, and immunostimulants in biomedical applications because they bias toward Th1 immunity. Their immunogenicity is explained by their high molecular mass (8 MDa), complex quaternary structure, and heterogeneous glycosylations, which are multiligand of innate immune receptors such as the Toll-like receptor 4 (TLR4) and the mannose receptor (MR). These receptors regulate the proinflammatory response, the endocytosis, and the intracellular processing of their agonists by antigen-presenting cells (APCs), following the proteasomal and/or lysosomal pathways, which generate peptides that bind to the major histocompatibility complex class I (MHC-I) and/or class II (MHC-II), activating adaptive immunity. Proteins that are slowly processed by APCs elicit stronger immune responses; nevertheless, most studies use model glycoproteins, such as Ovalbumin (OVA, 45 kDa), and not complex multiligand glycoproteins. We hypothesize that: "Hemocyanins, as exogenous complex glycoproteins, are slowly processed by both the proteasomal and lysosomal pathways, modulating their proinflammatory response". As murine APCs we used the JAWS-II cell line and bone marrow-derived dendritic cells. Furthermore, we used hemocyanins from *Megathura crenulata* (KLH) and *Concholepas concholepas* (CCH). Analyses of hemocyanin processing by immunoblot revealed fragments of >250 kDa after 96 hours of stimulation; in contrast, OVA was completely degraded. Similarly, hemocyanins promoted cytokine secretion after 96 hours of stimulation, as demonstrated by ELISA and flow cytometry; pharmacological inhibitors of the proteasome and lysosomal degradation impaired IL-6 and IL-12p40 secretion. These results suggest a correlation between the proinflammatory response and the slow processing of hemocyanins which contributes to explain their Th1 immunogenicity.

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54. Cholesterol-related lncRNAs as response predictors of atorvastatin treatment in Chilean patients with hypercholesterolemia. Isis Paez, Yalena Prado, Pía Loren, Carmen G. Ubilla, Nelia Rodríguez, Luis A. Salazar (luis.salazar@ufrontera.cl). Center of Molecular Biology and Pharmacogenetics, Department of Basic Sciences, Faculty of Medicine, Universidad de La Frontera, 4811230 Temuco, Chile.
Statins are currently the treatment of choice for hypercholesterolemia. However, wide interindividual variability has been observed in the response to treatment. Recent studies have reported the role of Long Non-Coding RNAs (lncRNAs) in the metabolism of lipids; nevertheless, there are few 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 six lncRNAs (ARSR, CHROME, LASER, RP1-13D10.2, MANTIS and lncHR1) associated with genes involved in cholesterol homeostasis in leukocyte cells of hypercholesterolemic patients after treatment with atorvastatin and compare them with levels in subjects with normal cholesterol levels. A secondary aim was to assess the levels of expression in monocytic THP-1 cells differentiated to macrophages. The study included 20 subjects with normal cholesterol (NC) levels and 20 individuals with hypercholesterolemia (HC). The HC patients were treated with atorvastatin (20 mg/day/4 weeks). THP-1 cells were differentiated to macrophages with PMA and treated with different doses of atorvastatin for 24 hours. Expression of lncRNAs was determined by RT-qPCR. The lncRNAs RP1-13D10.2 ($p<0.0001$), MANTIS ($p=0.0013$) and lncHR1 ($p<0.0001$) presented increased expression in HC subjects compared with NC subjects. Furthermore, atorvastatin had a negative regulatory effect on the expression of lncHR1 ($p<0.0001$) in HC subjects after treatment while increasing the expression of lncRNAs ARSR and CHROME ($p < 0.0001$) upon completion of treatment. LASER did not show significant differences among the groups ($p = 0.50$). In vitro, all the lncRNAs showed significant differences in expression after atorvastatin treatment. Our findings show that the lncRNAs tested present differential expression in HC patients and play a role in the variability reported in the response to atorvastatin treatment. Further research is needed to clarify the biological impact of these lncRNAs on cholesterol homeostasis and treatment with statins.
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55. Immune response characterization of a new formulation of a vaccine candidate against *L. intracellularis*. Santiago Salazar[1] (sasalazar@udec.cl), María Francisca Starck[1], Jorge R. Toledo[1], Álvaro Ruiz[2], Milton F. Villegas[1], Raquel Montesino[1]. [1]Biotechnology and Biopharmaceuticals Laboratory, Pathophysiology Department; School of Biological Sciences. Universidad de Concepción. Victor Lamas 1290, P.O. Box 160C, Concepción, Chile. [2] Pathology and Preventive Medicine Department, School of Veterinary Sciences. Universidad de Concepción, Avenida Vicente Méndez 595, Chillan, Chile.

Conventional vaccines approved to counteract *Lawsonia intracellularis,* the etiological agent of Porcine Proliferative Enteropathy (PPE), have practical and economic limitations avoiding the development of effective vaccination strategies to control this disease. Therefore, new vaccines are needed to face this problem that causes significant losses in the swine industry. In this study, we evaluated the safety and immune response induced by a new subunit vaccine candidate. We produced three recombinants *L. intracellularis* antigens as inclusion bodies in *E. coli*. Antigens were emulsified in the water-in-oil adjuvant Montanide ISA 660 VG. Immune response in pigs was evaluated by indirect and sandwich ELISAs, qPCR, and flow cytometry. The vaccine candidate's safety was assessed by histopathology and by monitoring the clinical behavior of animals. The vaccine candidate induced a significant immune response in inoculated pigs compared to the control groups. Humoral response rapidly increased one week after the booster in vaccinated pigs at doses of 100 and 200 µg and persisted until the end of the experiment (week 14). The dose of 200 µg significantly activated cellular response measured by transcriptional and translational levels of cytokines. Cell proliferation assay upon specific antigen stimulation revealed an increasing trend of lymphocytes T $CD4^+$ at the same dose. Animals gained weight constantly and showed proper clinical behavior during immunization assays. This research demonstrated the immunological robustness of this new subunit vaccine candidate against PPE. These promising results are encouraging to continue developing the formulation of the novel vaccine candidate to expand the available arsenal to combat PPE.

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56. Colorectal cancer screening through the detection of biomarkers in wastewater.
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Colorectal cancer (CRC) is the most frequent malignant neoplasm of the digestive tract, being one of the main causes of morbidity and mortality worldwide. There are various pathways by which CRC can be generated and from the altered genes in these is that specific biomarkers are released for the detection of this cancer. These can be detected in various bodily fluids, such as blood and feces, the second is possible thanks to the desquamation of tumor cells derived from the tumor mass that grows into the lumen of the colon. Due to tumor desquamation, it is possible to detect it in wastewater, which allows us to study and analyze specific human biomarkers excreted metabolically, being able to obtain epidemiological information on public health, habits, well-being, among others. Therefore, in this study we sought to detect the expression of 14 CRC mRNA biomarkers, such as MSH2, SEPT9, CEACAM5 and ITGA6, in wastewater samples from Concepcion in the Biobio region. In the first instance, we observe the increase expression of 4 genes and the decrease of 9 genes associated with CRC through RT-qPCR of three different cell lines. On the other hand, it was possible to detect by RT-qPCR most biomarkers in wastewater samples, comparing its expression with a control population. Together, these results suggest, that some of these biomarkers can be used for the early prediction and diagnostic of CRC, allowing the creation of a panel of specific biomarkers for the detection of this cancer at a population level.

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57. Extracellular vesicles from Caveolin-1-expressing B16F10 melanoma cells enhance *in vitro* vascular network formation by endothelial cells.

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Caveolin-1 (CAV1) is a membrane protein that promotes cancer cell migration, invasion, and metastasis in a manner dependent on CAV1 phosphorylation on tyrosine-14 (Y14). Additionally, extracellular vesicles (EVs) released by metastatic cancer cells promote migration and invasion of less aggressive recipient cells in a CAV1-dependent manner. However, the relevance of CAV1 phosphorylation in EV-stimulated events remains to be determined. Here we used B16F10 melanoma cells over-expressing CAV1 as a model to determine how the EV protein content was affected by tyrosine-14 phosphorylation and how these EVs modulated the angiogenic potential of endothelial cells. EVs were isolated by ultracentrifugation from B16F10(Mock), B16F10(CAV1), phospho-mimetic B16F10(CAV1/Y14E), as well as phospho-null B16F10 (CAV1/Y14F) cells. EVs were characterized using Nanotracking Analysis and Western blotting. The protein content of these EVs was further analyzed using shotgun proteomics. The biological effects of EVs in EA.hy926 cells were determined using an *in vitro* angiogenesis model. EVs of B16F10 cells over- expressing wild-type CAV1 and phospho-mimetic CAV1(Y14E) contain CAV1 and other proteins linked to signaling pathways associated with cell adhesion, migration, and angiogenesis. Moreover, these EVs, but not EVs from CAV1(Y14F) or Mock cells, increased vascular network formation *in vitro* by endothelial EA.hy926 cells. In conclusion, CAV1, and especially its phosphorylation at tyrosine-14, is implicated in modulating the content of EVs from B16F10 melanoma cells, thereby favoring the formation of linked networks by endothelial cells. Therefore, identifying the mechanisms by which EVs containing CAV1 promote effects that relate to angiogenesis in tumor development may be of great potential therapeutic interest.

58. B-cell lymphoma protein 6: a novel transcription factor involved in cardiac hypertrophy revealed through human transcriptional regulatory networks. Juan Francisco Silva-Agüero[1] (juan.silva.a@ug.uchile.cl), Víctor Aliaga-Tobar[2,3], Erik Lopez-Gallardo[1], Leslye Venegas- Zamora[1], Úrsula Zúñiga-Cuevas[1], Fernanda Fredericksen[1,2,4], Fernanda Zapata-Neweu[1], Mauricio Latorre[2,3,4,5], Valentina Parra[1,2]. [1]Department of Biochemistry and Molecular Biology and Advanced Center for Chronic Diseases (ACCDiS), Faculty of Chemical and Pharmaceutical Sciences, Universidad de Chile, Santiago, 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 Bioingeniería; Instituto de Ciencias de la Ingeniería; Universidad de O'Higgins, Rancagua, Chile. [4]Millenium Institute Center for Genome Regulation (MiCGR), ANID Millennium Science Initiative Program (ICN2021_044), Chile. [5]Laboratorio de Bioinformática y Expresión Génica, INTA, Universidad de Chile, Santiago, Chile.

Heart failure (HF) is a complex condition where the heart cannot pump enough blood to the body. HF onset precedes cardiac hypertrophy (CH), an adaptative response to increased workload characterized by cardiomyocyte enlargement. Cardiac stressors, such as Norepinephrine (NE), activate a global transcriptional response of different signaling cascades of master transcription factors (TFs, e.g., NFAT, GATA4, MEF2). Using a systems biology approach, we build a transcriptional regulatory network (TRN) activated by HF. Interestingly, the network contains several TF involved in CH/HF in addition to uncharacterized factors, thus selecting BCL6 as a potential new regulator. To evaluate BCL6 response under CH conditions, we treated neonatal rat cardiomyocytes (NRVM) with NE. We observed increased BCL6 mRNA and protein levels that correlated with the overexpression of hypertrophy marker (ANP and BNP). Additionally, NE stimulation increased BCL6 nuclear distribution consistent with cardiomyocyte hypertrophic growth. Furthermore, BCL6 knockdown on NE-treated NVRM ameliorated the overexpression of ANP and BNP and inhibited cardiomyocyte hypertrophic growth. BCL6 knockdown also recovered mitochondrial fragmentation and upregulated Mitofusin-2 protein levels, both characteristics of the metabolic switching observed in CH. Further, the BCL6 inhibitor FX-1 ameliorated ANP overexpression. To our knowledge, this is the first report showing a TRN activated by HF, a model capable of identifying new TF involved in CH. Finally, our results suggest that BCL6 may exert a critical pro-hypertrophic role. Future studies will be needed to evaluate BCL6 pro-hypertrophic mechanisms, potential therapeutic role on HF, and additional potential targets derived from HF-activated TRN.

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59. Study and Optimization of a Post-Harvest Treatment for Rosa Mosqueta to Maximize Anthocyanin Content. Brenda Soto (bsoto2018@udec.cl), Marcell Gatica, Amparo Uribe, Juan Román. Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile.

Anthocyanins, the largest group of plant pigments responsible for the red, purple, and blue hues of various plant tissues, play a crucial role in plant defense mechanisms against biotic and abiotic stress. Their consumption is linked to numerous health benefits, including reduced cardiovascular disease risk, enhanced cognitive function, and potential anticancer properties. Rosa mosqueta fruits, rich in anthocyanins, often lose their nutritional value due to factors like light, heat, and post-harvest processing. Despite Chile's abundant Rosa mosqueta cultivation, there's a pressing need to optimize post-harvest handling to retain the fruit's benefits. In this study, fruits were collected from a local farm in Concepción in April. A 3^2 experimental design was implemented considering two primary variables: storage time and temperature. The storage times were set at 5, 10, and 15 days, while temperatures were determined at 15°C, 4°C, and -20°C. The key metrics assessed included content levels of anthocyanins, phenolic compounds, antioxidant activity, and vitamin C. The preliminary results revealed that the total polyphenol content averaged 2.5gEAG/100g of fruit. The antiradical power (ARP) yielded an average of 300 umol/g of fruit, while the antioxidant activity was determined at 7.4 gEqTrolox/100g of fruit. Current work focuses on profiling vitamin C and anthocyanins in the fruit subjected to various treatments. The primary objective is to identify the operational condition that maximizes the content of bioactive compounds, thereby providing an optimized storage method and post-harvest treatment for enhanced preservation of anthocyanins in rosa mosqueta.

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60. Insight on the interaction of *agmatinase-like protein* (ALP) with Mn^{2+} cofactor.

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Agmatine is the decarboxylated product of L-arginine, this amine has hypoglycemic, anticonvulsant, antineurotoxic and antidepressant effects in humans. Our laboratory identified a new protein present in human and rodent brain that hydrolyzes agmatine in putrescine and urea, we call it *agmatinase-like protein* (ALP). ALP differs in its amino acid sequence from known prokaryotic agmatinases and does not contain typical residues that interact with the Mn^{2+} activator metal. We have generated a comparative structural model, considering the very low sequence similarity (30-38%) between ALP and the crystal structures of prokaryotic agmatinases, and we have proposed new ligands for the Mn^{2+} cofactor. In this work, we have generated three simple mutants of the residues proposed as ligands of Mn^{2+} ions: N213A, Q215A and D217A. The ALP-mutants did not present significant differences in the specific activity with respect to wild-type enzyme. Interestingly, the K_m for agmatine decreased between 50-55% in the ALP-mutants and the activation constant for Mn^{2+} decreased in an order of magnitude with respect to wild-type enzyme (this constant is related with the K_d of enzyme-metal complex). For ALP and other ureohydrolases, the incubating to 60°C with Mn^{2+} produce a hyperactivation of the enzyme and this phenomenon was maintained in the mutants. The kinetic effects generated by the alanine replacement in each mutant were similar, in all three cases the interaction with the substrate and with the Mn^{2+} cofactor is favored.

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61. Unraveling the Dynamics of SARS-CoV-2-PLpro Interaction with its substrate Ubiquitin-AMC. Jorge L. Valdes-Albuernes (jlvaldes1994@gmail.com), José L. Velásquez-Libera, Julio Caballero. Departamento de Bioinformática, Centro de Bioinformática, Simulación y Modelado (CBSM), Facultad de Ingeniería, Universidad de Talca.

The COVID-19 pandemic, caused by the zoonotic coronavirus SARS-CoV-2, has resulted in hundreds of millions of infected individuals and millions of deaths. Among the identified target proteins in this virus, the papain-like protease (PLpro), is involved in the infection and replication mechanisms. Additionally, it is engaged in antagonistic mechanisms to evade the host's immune system. The molecular structure of PLpro shares similarities with human deubiquitinating enzymes of the ubiquitin-specific protease family. It consists of four distinct subdomains: the "thumb", the "fingers", the "palm" (where the protein's active site is located), and the ubiquitin-like (Ubl) domain. PLpro of coronavirus contains the conserved catalytic triad Cys- His-Asp at the active site. In this study, we investigated the SARS-CoV2-PLpro system complexed with the ubiquitin-AMC (7-amino-4-methylcoumarin) substrate. Enzyme-substrate structural models were generated using comparative modeling through MODELLER. These models were subsequently employed for molecular dynamics simulations using the AMBER18 suite. As a result, trajectories representing the system's temporal evolution were acquired. Utilizing these trajectories, a variety of analyses were conducted. These encompassed tracking distances and geometries of specific catalytically relevant groups, performing Interaction Fingerprint (IFP) calculations, relevant coordinate computations, and clustering techniques (PCA). This methodology led to the identification of distinct conformational groups within the enzyme-substrate complex. We elucidated the essential changes between these groups and pinpointed those contributing to the initiation of catalytic activity. This comprehensive approach enhances our comprehension of PLpro-ubiquitin-AMC interactions, a crucial insight for the rational design of drugs targeting this protease.

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62.Alcohol acyltransferases and their importance in aroma production in developing *Fragaria chiloensis* fruit. Felipe Valenzuela-Riffo (felvalenzuela@utalca.cl); Joselin Guajardo; M. Alejandra Moya-León; Raúl Herrera. Laboratorio de Fisiología Vegetal y Genética Molecular, Instituto de Ciencias Biológicas, Universidad de Talca, Talca, Chile.

Alcohol acyltransferases (AATs) catalyze the condensation of Acyl-CoA and alcohols to synthesize esters. Esters are volatile compounds, exuded by flowers and fruits, which influence consumer interest. Previously, *FcAAT1* was isolated and characterized from *Fragaria chiloensis* fruit, showing an expression pattern that increments as the fruit ripens. At early stages of fruit development few esters were detected however *FcAAT1* transcripts were not accumulated at this stage, therefore a genome-wide analysis was performed to find the missing genes. Five different *FcAAT*s were identified in the *F. chiloensis*´s genome, including *FcAAT1*. Interestingly they belong to different phylogenic groups. Substantial differences were observed at the sequence level among the different FcAATs, nevertheless, the five sequences contained the alcohol acyl transferase domain and two other conserved domains of the family: HxxxD and D(N)F(V)GWG. At the structural level, by means of comparative modeling, it was observed that the protein structures differ in the shape and size of their solvent channels which is determinant for substrate selection and could define the type of ester synthesized. These differences could explain the different set of esters produced by the fruit. The level of transcripts determined by qRT-PCR for the five *FcAATs* display different accumulation patterns along fruit development and ripening, and the expression profile is coincident with FPKM values from RNAseq data. This information is of utmost importance to explain the production of esters during development and ripening of *F. chiloensis* fruit.

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63. Optimization of the expression of soluble acuaporin Z in *Escherichia coli.* Vera-Oñate Valentina (vvera2016@udec.cl), Beratto-Ramos Ángelo, Martínez-Oyanedel José. Laboratorio de Biofísica Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.

The water transport across membranes is essential for the regulation of homeostasis in all living organisms. This physiological process occurs because the presence of pores or channels formed by membrane proteins known as porins, which quickly respond to sudden changes in extracellular osmolality maintaining hydroelectrolyte balance. Aquaporin Z (AqpZ) is a tetrameric protein expressed in *Escherichia coli*. Currently, various studies aim to incorporate Aquaporins Z to filtration membranes for their application in the treatment of water for human consumption, however, their production and obtaining from *Escherichia coli* cultures is limited by low expression levels and formation of inclusion bodies when protein is expressed at high concentrations. In the present work, the expression of AqpZ in soluble form was optimized from a recombinant strain of E. coli, using variation of culture conditions.
The production of soluble AqpZ was evaluated by SDS-PAGE on 12% polyacrylamide gels, stained with Coomassie blue, scanned and analyzed by densitometry with the ImageJ program.
The results indicate that the optimal bacterial culture conditions for soluble protein expression are: induction until OD_{600} 0.6 then grown at 27 °C using 0.5 mM IPTG for 16 hours. These conditions allow obtaining up to 14 mg of protein per liter of culture.
Additionally, the production of AqpZ by auto induction was studied and compared using the previously optimized conditions, this increases the yield to around 20 mg protein per liter culture.

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64. Purification of GFP-Histatin-1 and assessment of biological activity. Monserrat Videla[1,2] (monserrat.videla@ug.uchile.cl), Rodrigo Rivera[1], Carlos Mateluna[2], Vicente A. Torres[2], Mauricio Baez[1]. [1]Laboratory of Biochemistry, Faculty of Chemical and Pharmaceutical Sciences, Universidad de Chile; [2]Laboratory of Cellular Biology, Faculty of Dentistry, Universidad de Chile, Advanced Center for Chronic Diseases, and Millennium Institute on Immunology and Immunotherapy.

Histatins are a family of histidine-rich, low molecular weight proteins, expressed in human saliva. From this group, Histatin-1 is a peptide that promotes wound healing in different tissues, by triggering a subset of signaling pathways associated with cell migration. However, the mechanism of internalization of Histatin-1 and its subsequent endocytosis, remain poorly understood. With the objective of elucidating this process, we designed and purified a GFP-Histatin-1 fusion protein, in order to allow subcellular localization of this construct by fluorescence. To facilitate the purification of the GFP-Histatin-1 fusion protein, a poly-histidine sequence, fused to the GroEs chaperone subunit, was added at the carboxyl-terminal end. Separation of GFP-Histatin-1 from GroEL-His was performed by proteolytic cleavage using Usp2-cc protease. GFP-Histatin-1 purification was accomplished via nickel affinity chromatography, whereby two fluorescent species with different molecular weights were obtained, suggesting partial endogenous degradation of the expressed peptide. The digestion product of Usp2-cc (GFP-Histatin-1) was used in a Transwell migration assay to assess biological activity. Unfortunately, unlike non-tagged Histatin-1 (commercially available), GFP-Histatin-1 failed to induce endothelial cell migration. The absence of the expected cellular responses might be due to interactions between GFP and Histatin-1, which prompt us to explore new approaches for labeling and tracing Histatin-1 in cell culture models.

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65. Structural and evolutionary analysis of gram-positive bacterial pilins. Pablo Villalobos[1] (pavillalobos@u.uchile.cl), J. Andrés Rivas Pardo[2], Víctor Castro-Fernández[1]. [1]Laboratorio de Bioquímica y Biología Molecular, Departamento de Biología, Facultad de Ciencias, Santiago, Chile. [2]Mechano-Biology Group, Microbe Genomics Lab, Center for Genomics and Bioinformatics, Universidad Mayor, Santiago, Chile.

Pathogens bacteria anchor to their host cell through their pilli, proteins (pilins) that must resist considerable mechanical stresses caused by the host. Gram-positive pilli comprises repeated pilins subunits, proteins that display an unusual bond between an Lys-Asn side chains also known as isopeptide bond. This covalent link connects two beta strands of the protein domain, preventing the unfolding. The mechanical resistance of the gram-positive Spy0128, the pilin from the human pathogen *Streptococcus pyogenes,*has been assessed through atomic-force spectroscopy. Force experiments showed that Spy0128 is practically inextensible. In this work, we aim to understand the evolution of mechanical resistance of Spy0128 and related proteins, implementing phylogenetic, molecular modeling, and molecular dynamics studies. The inferred phylogeny shows Spy0128 pilin in a branch composed mainly of proteins from Streptococcus spp. diverging from a common ancestor (AncCS) with sequences from Clostridium spp. among other gram-positive bacteria. Closer to the AncCS we observed a sequence of another common human pathogen, *Clostridium difficile* (Cdi0128). All of those sequences, including the reconstructed ancestral sequence of AncCS, possess the Lys-Asn motif, a signature of isopeptide bond formation. To validate this idea, we employed AlphaFold to predict the structural arrangement of the sequences from AncCs, Cdi0128, and Mcc804621. The modeling indicated the presence of the characteristic immunoglobulin fold of Spy0128, maintaining the Lys-Asn isopeptide bond. The stability of the architecture was also confirmed in preliminary molecular dynamics studies. Altogether those results suggest that isopeptide bond formation is a signature in Spy0128 homologs, conferring mechanical resistance to its structure.

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66. Expression of IL-8 in whole tissue sections of human gastric tumors and its association with inflammation. Ignacio González[1,2]*, Leyla Jaupi[1,2]*, Karina Cereceda[1], Antonia Geisse[1], Constanza Cárcamo[1], Roxana González-Stegmaier[1] and Franz Villaroel-Espindola[1] (franz.villarroel@falp.org). [1]Translational Medicine Laboratory, Instituto Oncológico Fundación Arturo López Pérez, Santiago, Chile. [2]Medical Technology School, Universidad San Sebastián. Santiago, Chile.

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Gastric cancer (GC) is a multifactorial disease related to several risk factors, such as *Helicobacter pylori (H. pylori)* infection, high consumption of alcohol and salt, smoking, and some genetic variants. Two major histological GC-subtypes are described, Intestinal and Diffuse, and both of them have been associated to different oncogenic pathways. In the Intestinal-subtype, once the normal epithelium becomes abnormal after being infected by *H. pylori*, the oncogenic cascade progress slowly, including several degrees of local inflammation and cytokines expression. High levels of IL-8 in serum correlate with poor-outcome in cancer patients, but its value as tissue biomarker in GC is still unclear. This work aimed to evaluate the spatial IL-8 distribution within the tumor microenvironment in surgical tumor resections. Sixteen whole tumor sections with a diagnosis of an Intestinal-subtype of gastric adenocarcinoma were assessed using multiplexed immunofluorescence. IL-8 was simultaneously detected within immune cells (CD45+) or tumor cells (Cytokeratin, CK+). In addition, the tissue-infiltration with CD8+ cytotoxic T-lymphocytes and CD11b+ myeloid cells were estimated and correlated with IL-8 presence. Tissue segmentation and cell phenotyping showed a wide range of immune-infiltration and positivity for IL-8 in tumor cells. The presence of IL-8+ tumor cells seemed not to be significantly related to the tumor stage, *H. pylori* status or abundance of immune cells. These results confirmed the complex biological role and clinical significance of IL-8 in GC, and allow us to suggest that depending on the cell type expressing IL-8; it may display inflammatory and protumor properties.

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67. Potential immunostimulant of *Salmo salar*'s HMGB1 and derived peptides. Milton Villegas (miltonfvillegas@udec.cl), Aleikar Vásquez, Carolina Muñoz, Crisleri Carrera, Leonardo Ortega, Santiago Salazar, Jannel Acosta. Biotechnology and Biopharmaceuticals Laboratory, Pathophysiology Department; School of Biological Sciences. Universidad de Concepción, Concepción, Chile.

This study used molecular docking techniques to design a series of peptides derived from *Salmo salar*'s truncated HMGB1 acidic tail, with a focus on their potential as TLR5 agonists. To establish a baseline for comparison, a reference peptide (SsOri) with an authentic N-terminal acidic tail was included. The interaction of these peptides with the TLR5 ectodomain was methodically assessed using scoring functions. This was complemented by circular dichroism analysis of selected peptides (6W and 11W), which preceded experimental assays aimed at evaluating their agonistic potential, particularly regarding the activation and nuclear translocation of the p65 NF-kB subunit. Notably, HeLa cells expressing *Salmo salar* TLR5 exhibited heightened p65 phosphorylation comparable to flagellin-induced levels when compared with 11W, thereby highlighting the engagement of TLR5 and initiation of immune responses. Moreover, the study extended its scope to encompass the production of recombinant HMGB1 protein (rHMGB1) from *Salmo salar*. This process involved the synthesis of rHMGB1 within *Escherichia coli* and subsequent purification to approximately 50% purity using FPLC. By employing rHMGB1 and the 11W peptide as distinct treatments, the study probed the impact on immune factors (TNF, IFN-γ, MyD88, EF1, IL1 and IL8) over different time intervals (3, 6, and 12 hours). It is noteworthy that a significant increase in the selected immune factors was observed in rHMGB1 and 11W. This underscores the potential of HMGB1 and its derived peptide as immunostimulant, providing a comprehensive approach to enhancing immune responses in salmonids.

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68. Effects of knots in protein degradation by ATP-dependent proteases using GFP-MJ0366 fusion protein constructs.

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ATP-dependent proteases translocate proteins through a narrow pore for their con- trolled degradation. However, how a protein containing a knotted topology affects this process remains unknown at a molecular level. Our experimental data suggest that a substrate-fused shallow 3_1 knot (GFP-MJ0366 construct) could impair protein translocation at standard prote- olysis mediated by archetypal AAA+ ClpXP protease. The degradation could lead to the release of partially processed products that may be harmful for the cell or possess new biological ac- tivities. This knot gives some kind of protective degree that may be related to the length of the fusion linker (San Martín A et al., 2017). In this work we implemented a Cα coarse-grained pro- tein simulations to account for the above translocation process based on an open source project named OpenMM, coded in pure Python in a Jupyter Notebook. In addition to the simulation algorithm, we developed two extra modules: an implicit pore to study the protein translocation and a constant-force pulling potentials to make the protein go through the pore. We simulated GFP-MJ0366 fusion proteins with different linkers sizes ranging from 17 to 117 amino acids. Early results would confirm the experimental behavior, where linkers of more than 36 residues between the MJ0366's knot core and GFP would allow the complete unfolding and degradation of the substrate. Nevertheless, it is still necessary to analyze the effect of all linker sizes, in- crease the number of replicas and better tune the pore potential in the dynamics.

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69. Study of ADP-ribosylation of de novo synthesized histones H3 and H4. Patricio Zapata (pzapatac1@correo.uss.cl) & 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.

ADP-ribosylation (ADPr) of histones involves the covalent transfer of poly-ADP-ribose (PAR), catalyzed by PARPs, and removed by PARGs. This mark plays a role in DNA damage. In the cytoplasm, this modification is observed in histones and it occurs during the histone maturation process, following their synthesis. H3 and H4 are ADP-ribosylated when these two histones are not associated yet with each other. When they come together through the "histone fold" to form an H3-H4 dimer, both histones lose the ADPr. This study investigates the enzymes involved in the formation of PARs prior to the formation of the H3-H4 dimer in newly synthesized histones, as well as the function of this mark. Identification of the PARP was carried out through a shRNA screening against PARP enzymes in HeLa cells, combined with Western blot assays and corroborated through in vitro experiments. The results suggest that histones may be undergoing modification by PARP enzymes, and the presence of this PTM could be altering their proper processing. ADPr in newly synthesized histones is of great importance as it could be involved in the formation of the H3-H4 dimer, a key component of nucleosome formation, the fundamental unit of chromatin. The maintenance of chromatin is essential to preserve genetic material and protect cells from genomic instability, one of the main causes of aging and various types of cancer.

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70. hnRNP A1, hnRNP K and HuR modulate HTLV-1 IRES-mediated translation initiation.

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The human T-cell leukemia virus type 1 (HTLV-1) is a complex retrovirus that causes adult T leukemia and HTLV-associated myelopathy/tropical spastic paraparesis. HTLV-1 transcribes sense and antisense mRNAs.The sense, full-length mRNA, can initiate translation using a cap-dependent mechanism or an internal ribosome entry site (IRES), the HTLV-1 IRES. The HTLV-1 IRES enables the recruitment of the ribosome in a 5'cap-independent fashion. In general, the activity of the retroviral-IRESs requires IRES-transacting factors (ITAF). hnRNP A1, hnRNP K, and HuR are known ITAFs for the HIV-1 IRES. Of these proteins, only hnRNPA1 has been reported to promote HTLV-1 IRES activity. Thus, using a dual luciferase bicistronic mRNA construct harboring the 5'leaders of the sense HTLV-1 mRNA in its intercistronic space, we evaluate in mammalian cells the role of hnRNP A1, hnRNPK, and HuR, alone or in combination, as regulators of the HTLV-1 IRES function. Our findings suggest that hnRNP A1 and hnRNP K promote, while HuR represses HTLV-1 IRES-mediated translation initiation. Interestingly, when combined with HuR, hnRNP A1 and hnRNP K reverse the repression in IRES-mediated translation initiation. We conclude that HTLV-1 IRES activity relies on the combined effect of its ITAFs.

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71. Impact of hnRNP A1, hnRNP K, and HuR on sHBZ IRES-mediated translation initiation. Diego Guzmán (diegoguzmancornejo@gmail.com), Camila Blanlot, Brenda López Ulloa, and Marcelo López-Lastra.
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The human T-cell leukemia virus type 1 (HTLV-1) is a complex retrovirus that causes adult T leukemia (ATL) and HTLV-associated myelopathy/tropical spastic paraparesis. HTLV-1 transcribes sense and antisense mRNAs. The HTLV-1 antisense mRNA encodes for the HTLV-1 bZIP factor (HBZ) protein. Interestingly, HBZ is expressed in cells in all cases of ATL and is directly associated with virus pathogenicity. The spliced isoform of HBZ (sHBZ) is encoded by the spliced hbz (shbz) mRNA. Translation initiation of the shbz mRNA is exclusively mediated by an internal ribosome entry site (IRES), the sHBZ IRES, located in the mRNAs 5'leader. As the activity of most retroviral-IRESs is modulated by IRES-transacting factors (ITAFs), we wondered whether the sHBZ IRES was also regulated by ITAFs. Using bicistronic vectors harboring the 5'leader of the spliced antisense (sHBZ) mRNAs in their intercistronic space, we evaluate the role of hnRNP A1, hnRNPK, and HuR, known ITAFs for other retroviral IRESs, as regulators of the sHBZ IRES function. Our findings suggest that all three evaluated proteins act as ITAFs regulating the sHBZ IRES activity.

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72. Improved therapeutic response through antagonism of the A2B adenosine receptor in glioblastoma stem like cells. Pamela Silva[1] (pamesilvalv@gmail.com), Patricio Saldivia[1]; Giovanna Navarro-Martínez[1]; Diego Carrillo [1,2]; Claudia Quezada [1,2]. [1]Laboratorio de Biología Tumoral, Instituto de Bioquímica, Facultad de Ciencias, Universidad Austral de Chile. [2]Instituto Milenio de Inmunología e Inmunoterapia

Background. Glioma stem like cells (GSCs) subpopulation has been identified in glioblastoma and plays a key role in tumor resistance to conventional therapies. GSCs are classified as proneural or mesenchymal subtypes based on gene signatures, metabolic phenotypes and tumor resistance performance. GSC produce large amount of extracellular adenosine levels, especially under hypoxic environments, activating the low affinity A2B receptor (A2BAR). Studies in our laboratory have shown that pharmacological blockage of A2BAR decreased migratory and invasive activity of GSC in vitro, which may be related to potential anti-tumoral action. For this reason, we examined the effects of A2BAR modulation in ROS production, proneural-mesenchymal transition and response to the alkylating drug temozolomide (TMZ).
Methodology. Mes-GSC were treated with 50nm of MRS1754, A2BAR antagonist, in presence or not of 400uM TMZ and cell viability were evaluated by MTS and AnnexinV/PI assay. ROS production were examined by fluorescent dye DCFDA and transition markers were evaluated by flow cytometry assay.
Results. Antagonism of A2BAR chemosensitize GSCs to temozolomide treatment through an increase intracellular ROS production. Flow cytometry analysis showed a reduced CD44+ population under MRS1754 treatment as well as in *knock out*-A2BAR-GSCs. In these cells, an increase in the CD133+ population also was observed.
Conclusions. MRS1754 exhibits significant antitumor activity, induces apoptosis through the elevated production of reactive oxygen species. Regulation of A2BAR signaling may have clinical value as adjuvant in the glioblastoma treatment.

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73. Effect the cold shock protein CspA in long RNA unfolding mechanism. Cristian Valdebenito (cristian.valdebenito3@gmail.com), Rodrigo Rivera, Cayetana Zamorano, Mauricio Báez L. Laboratorio de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile.

The tridimensional structure and molecular dynamics of Ribonucleic acids play essential functions in the cell, ranging from the transfer of genetic information, the catalysis of chemical reactions, to the regulation of cellular processes. Currently, it is well known that a temperature downshift stabilizes secondary RNA structures and promotes the formation of misfolding RNA intermediates. Coherently, in mesophilic organisms a temperature downshift triggers a cold shock response in which a conserved group of RNA-chaperones, cold shock proteins (CSPs), dramatically increase their intracellular concentrations yielding a higher translation efficiency of mRNA. In this work, we use optical tweezers to determine how the presence of CspA affects the unfolding mechanism of a long RNA composed by 864 nucleotides which by comp utational prediction is proposed to form a one long initial helix of 283 bases-pairs when is mechanically perturbed at constant force. We measured how the time required to completely unfold the RNA change as we add crescent CspA concentration. Additionally, when this RNA is pulled at constant velocity presents a multiple unfolding transitions behavior coherent with the reported for similar systems, so additional experiments are going on to address the effect that CspA have in its unfolding mechanism. Together, this study aims to identify how this chaperon affect the structural dynamics of a long and continuous helix segment of RNA.

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