XLV ANNUAL MEETING

Chilean Society for Biochemistry and Molecular Biology

2022

XLV ANNUAL MEETING

CHILEAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY

November 22nd -25th, 2022

XLV ANNUAL MEETING CHILEAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY

DIRECTORY

President : Lorena Norambuena
Vice-president : Roxana Pincheira
Past-president : Luis Larrondo
Secretary : Julio Tapia
Treasurer : Luis Morales

DIRECTORS

North : Ricardo Tejos
Santiago : Clara Quiroga
Santiago : Mauricio Budini
Talca : Simón Ruiz
Concepción : Matías Hepp
Valdivia : Francisco Salinas

SBBMCh ETHICS COMMITTEE

Ilona Concha
Ulrike Kemmerling Wels
Lorena Pizarro

ACCOUNT REVISION COMMITTEE

Javier Canales
Valentina Parra
Patricio Ramos

OPENING LECTURE

Running from the law: how to evade the genome sentinel Rho during stress

Irina Artsimovitch. Department of Microbiology and The Center for RNA Biology, The Ohio State University, Columbus, Ohio, USA

Irina Artsimovitch graduated from Moscow State University (Russia) with a M.S. Degree in Bioorganic Chemistry. She received her Ph.D. in Microbiology from the University of Tennessee-Memphis and was trained as a postdoc in the laboratory of Robert Landick at the University of Wisconsin-Madison. In 2001, she joined the Microbiology Department at the Ohio State University as Assistant Professor. She is currently Arts and Sciences Distinguised Professor in Microbiology and Fellow of American Association for the Advancement of Science and of the American Academy of Microbiology. Her laboratory uses a range of experimental approaches to study RNA chain synthesis by multi-subunit RNA polymerases from bacteria and viruses, focusing on diverse catalytic reactions carried out by RNA polymerases, crosstalk between transcription and other processes, such as translation, DNA repair, and RNA folding, and mechanisms of accessory protein factors and small molecule ligands, including antibiotics and antivirals, that act upon RNA polymerases.

Abstract
Bacterial RNA helicase Rho is a genome sentinel that ensures that only “worthy” RNAs are transcribed. Rho blocks synthesis of antisense and xenogeneic RNAs, as well as mRNAs that contain early stop codons or ribosome-stalling signals. Textbooks state that Rho binds to nascent RNA, uses its motor activity to catch up with the transcribing RNA polymerase, and then releases RNAs that are not protected by ribosomes.

Realizing that this model cannot explain how Rho targets RNAs that are still being made, we demonstrated that Rho rides along with RNA polymerase instead and inactivates it allosterically. The revised model explains how Rho surveils the nascent RNAs, but raises a key question: how is indiscriminate termination avoided during translational stress or even slow growth? Using genetic selection, we identified Rho mutants that form long helical filaments, in which Rho hexameric rings are stacked on top of each other, akin to filaments of Rho homolog RecA. We showed that while these mutants form filaments under all conditions, filamentation of wild-type Rho is triggered by ADP and ppGpp, nucleotides whose levels increase during stress.

Our structural and biochemical data reveal that residue substitutions and ligands remodel the Rho ring to foster oligomerization. Filamentation is a widespread mechanism of adaptation to stress in all domains of life, and we hypothesize that Rho ring can readily transition between an active hexamer and inactive aggregates in response to cellula

SEVERO OCHOA CONFERENCE

Cell senescence in development, regeneration and cancer

Manuel Collado, Laboratory of Cell Senescence, Cancer and Aging, Health Research Institute of Santiago de Compostela, IDIS, Santiago de Compostela, Spain

Manuel Collado obtained his BSc and PhD from the Universidad Autonoma de Madrid (UAM). After his PhD, he moved first to London, UK, to work as a postdoc at the Ludwig Institute for Cancer Research at the St Mary's Branch, part of Imperial College School of Medicine, and then he moved to New York, USA, to join Memorial Sloan Kettering Cancer. He returned to Spain to join Manuel Serrano's lab, first at CNB and later on at CNIO, both in Madrid. After almost 11 years in Serrano's lab, Collado became group leader at the Health Research Institute of Santiago de Compostela, IDIS, where he leads the Cell Senescence, Cancer and Aging lab since 2012.

His work has been devoted mainly to the study of cell senescence where he has made high impact contributions such as the original description of oncogene-induced senescence in vivo (Nature 2005), the role of the Ink4/Arf locus as a barrier to cell reprogramming (Nature 2009), or the existence of developmentally-programmed cell senescence during embryogenesis (Cell 2013). Collado is a member of the Spanish Network on Cell Senescence and a steering committee member of the International Cell Senescence Association, ICSA.

Abstract
Cellular senescence is considered a stress response that limits the proliferation of damaged cells by establishing a permanent cell cycle arrest, providing a crucial antitumor defense mechanism for the organism. However, excessive production or impaired clearance of these cells can lead to their accumulation during aging with deleterious effects. Despite the potential negative side of cell senescence, its physiological role as a pro-regenerative and morphogenetic force has emerged recently after the identification of programmed cell senescence during embryogenesis and during wound healing. In our lab, we explored the conservation of developmental and tissue injury-induced senescence in a lower vertebrate showing complex regeneration, the zebrafish.
Although cancer cells manage to bypass the senescence response to proliferate without restriction, several antitumor treatments reactive this response. Chemotherapy of cancer

has traditionally been developed to induce cancer cell death, but we have now observed that when these treatments fail to kill cancer cells, they enter a state of cell senescence and remain metabolically active within the tumor. Senescent cancer cells secrete factors that can alter tumor microenvironment to promote secondary tumor growth and increase malignancy of cancer cells. For this reason, a novel anticancer strategy has recently been developed known as the “one-two punch” approach against cancer. In this strategy, senescence-inducing chemotherapies (the first punch) are combined with senescent specific cytotoxic drugs known as senolytics (the second punch). In our lab we have searched for novel senolytic drugs and identified a new family of compounds with senolytic activity that could be applied in cancer patients to develop more robust and definitive anticancer treatments.

OSVALDO CORI CONFERENCE

Epigenetic control of gene transcription during cell lineage commitment

Martín Montecino, Institute of Biomedical Sciences and Millennium Institute Center for Genome Regulation CRG, Faculty of Medicine and Faculty of Life Sciences, Universidad Andres Bello, Santiago, Chile

Martin Montecino graduated as a Biochemist from the University of Concepcion in 1989, obtained his MSc from the same institution in 1991, and received his PhD in Biomedical Sciences from the University of Massachusetts Medical School, USA, in 1996. He has worked in the field of epigenetic control of gene expression since becoming a graduate student and subsequently as an independent investigator at the University of Concepcion in 1997, at which he became Professor of Biochemistry and Molecular Biology in 2004. In 2010 he moved to the Andres Bello University, Santiago, to become Director of the Center for Biomedical Research which in 2018, became the Institute of Biomedical Sciences. During his career, he has authored and co-authored more that 180 publications and 21 book chapters, and has mentored 28 PhD students, 31 MSc and undergraduate students and 14 postdoctoral fellows. Additionally, he has directed and co-directed numerous nationally and internationally funded research grants in the field of gene regulation mechanisms, including as Deputy Director of the FONDAP Center for Genome Regulation.

Abstract

Pluripotent stem cells (PSCs) give rise pro-neural progenitors (NPCs) that therein differentiate to neurons and glial cells. In recent years, we have addressed fundamental epigenetic mechanisms that regulate gene expression programs allowing the establishment of pro-neural cells and those controlling subsequent neural-lineage commitment. Using NPCs obtained from induced pluripotent cells (hiPSCs) and global genomic approaches, we have mechanistically assessed the question of how the acquisition of a neural phenotype concomitantly involves silencing of gene programs associated with alternative lineages. Our data show that neural-engagement involves epigenetic silencing of non-neural gene programs (e.g. mesenchymal-osteogenic lineage). Hence, as the commitment of PSCs to the osteogenic lineage is mainly regulated by the bone master transcription factor RUNX2, differentiation of PSCs to NPCs requires silencing of the RUNX2 gene. Importantly, recent results indicate that RUNX2 also mediates critical stages during the

differentiation of NPC to astrocytes. This raises mechanistic questions about how the expression of the RUNX2 gene is initially repressed during differentiation of PSCs to NPCs and how RUNX2 expression is re-activated later during astrogliogenesis, without inducing the osteogenic lineage. Our results show that RUNX2 transcription is maintained silent in PSCs by inactivation of the osteogenic tissue-specific promoter P1. Moreover, that RUNX2 transcription during astrogliogenesis requires the activation of a second internal promoter P2, which is maintained in NPCs in an epigenetically bivalent condition that minimally transcribes RUNX2 mRNA (enrichment of the histone marks H3K4me3 and H3K27me3). This repressive profile then transitions to a transcriptionally pro-active condition (enrichment of H3K4me3 and H3K27ac marks) during the commitment of NPCs to astrocytes. Our analysis includes the role of specific enzymes that “write” and “erase” these epigenetic marks at the RUNX2-P2 promoter.

ANID/FONDECYT-1211026 and ANID/MILENIO-ICN2021_044

SYMPOSIUM
"Plant Defense"

Chair: Lorena Pizarro, Laboratorio de Inmunidad Vegetal, Instituto de Ciencias Agroalimentarias, Universidad de O'Higgins, San Fernando, Chile

I'm a plant biologist with a Ph.D. in Science from the "Molecular Biology, Cell Biology and Neuroscience" program at the Universidad de Chile. Currently, I am an Assistant Professor at the Instituto de Ciencias Agroalimentarias, Animales y ambientales (ICA3) from the Universidad de O'Higgins. My research is focused on Plant Immunity, describing defense responses in fruit trees and identifying the pattern recognition receptors that sense the pathogens. I have a broad background in plant biology, with specific training in cell biology and live-cell microscopy. I participate as Main Investigator in the "Anillo-O'Higgins Grant – ACT0190001" entitled "Deep insight into cherry plant defense responses to bacterial canker disease in a scenario of water restriction" and I lead two grants focused in the study plant immunity on stone fruit trees: "Peach (Prunus persica) pathogen recognition receptors (PRRs) and their role in pathogen resistance" (Fondecyt de Iniciación 2020 N°11200934); and "Activación de la inmunidad en frutales del género prunus para el potenciamiento de su tolerancia a enfermeddes fungosas y bacterianas"(PAI 77190027). With this research, I aim to promote sustainable strategies for plant protection in our agriculture.

1.- "Uncovering new roles for cytokinin in plant immunity"

Maya Bar, Department of Plant Pathology and Weed Research, Plant Protection Institute, Agricultural Research Organization, Volcani Institute, Rishon LeZion, Israel

The Bar lab researches plant immunity, investigating priming, biocontrol, and development-defense trade-offs. With a dual background in plant immunity and plant development, Dr. Maya Bar has published ~50 papers (~1400 citations, h-index 18).
Located at the Agricultural Research Organization- Volcani Institute in Israel, the Bar lab conducts basic and applied research of the plant immune system, investigating immunity priming, bio-stimulation, chemical and organismal biocontrol, development-defense trade-offs, and bio-sensing of plant disease. Main concepts in the lab include manipulation of immunity priming to generate plants with constitutively "ON" immune responses, resulting in broad spectrum resistance, and research into development-defense tradeoffs with the hope of "hacking" these processes to prevent yield loss. In the context of development-defense tradeoffs, we investigate the roles of plant developmental hormones, chiefly cytokinin, in plant-microbe interactions and plant immunity.

Abstract
The plant hormone cytokinin (CK) is an important developmental regulator, promoting morphogenesis and delaying differentiation and senescence. From developmental processes, to growth, to stress tolerance, CK is central in plant life. CK is known to induce host plant resistance to several classes of phytopathogens, with induced host immunity via salicylic acid signaling suggested to be the prevalent mechanism for this host resistance. Several classes of microbes can produce CKs, affecting the interaction with their plant hosts. Our lab is engaged in investigating several aspects of CK-mediated immunity and disease resistance.
Recently, we have uncovered a direct effect of CK on fungi. We found that CK directly inhibits the growth, development, and virulence of fungal phytopathogens, and can simultaneously promote energy production in phytopathogens in anticipation of plant infection. We investigated the roles of CK in shaping the plant host microbiome, examining the relationship between CK and the phyllosphere microbiome. We found that CK acts as a selective force in microbiome assembly, increasing richness, and promoting the presence of Bacilli. CK-mediated immunity was found to partially depend on the microbial community, and bacilli isolated from CK-rich plant genotypes induced plant immunity, and promoted disease resistance. Overall, our results indicate that host genotype and hormonal profiles can act as a strong selective force in microbiome assembly, underlying differential immunity profiles, and pathogen resistance as a result.
This work was supported by The Israel Science Foundation (1759/20).

2.- "Incidence of bacterial canker in sweet cherry orchards of the O'Higgins Region of Chile: Varietal susceptibility and genetic characteristics of the causal agent"

Boris Sagredo, Centro Regional Rayentué, Instituto de Investigaciones Agropecuarias (INIA), Rengo, Chile

Boris Sagredo is a biochemist from the University of Chile, has worked at the Agricultural Research Institute (INIA) since 1994. He completed his Cellular and Molecular Biology Ph.D. at the North Dakota State University (NDSU) in the USA. His lines of research are related with development of tools of genetic information based on DNA polymorphisms, aims to facilitate the selection task within the crops breeding process. He worked in the Potato Breeding Program of INIA at Osorno city during 10 years. Dr Sagredo represented Chile in the International Potato Genome Sequencing Consortium (PGSC). Since 2009 he has been working in the central zone of Chile supporting the sweet cherry, quinoa and cereal breeding programs. An important aspect of his research is the study of the resistance/susceptibility of crops to pests and diseases, which also involves to

study the populations of the pathogens. Always working in multidisciplinary groups in an associative manner, he has more than 48 high-impact scientific publications. He is an active member of the Chilean Genetic Society and the Chilean Plant Biology Society. He is currently Deputy Director of R + D of the Regional Research Center INIA-Rayentué of the O'Higgins Region of Chile.

Abstract

The sweet cherry (*Prunus avium* L.) Chilean industry has grown enormously during the last years, the area of planted production increased from 8.531 ha to 48.960 ha, during 2003-2021. At the global context, Chile is the 6th producer of sweet cherry of the world but it is the first exporter from the south hemisphere. Bacterial canker is the most important disease on cherry trees, caused by species of the Pseudomonas syringae complex. With poor control, cherry tree losses of up to 40% in young plants and 10 to 20% in commercial orchards can be reached. Host plant resistance is the most desirable element to develop integrated pest management strategies of control. The use of resistant plant reduces the use of pesticide that are expensive and sometime they represent a risk for the human and animals. In order to identify candidate sources of resistance to develop new varieties with reduced susceptibility, the incidence of bacterial canker among 23 varieties distributed in commercial sweet cherry orchards of the O'Higgins Region, was assessed. It was established that severity of bacterial canker mainly depends on the variety and occurrence of frost events, with 'Santina' and 'Bing' as the less and more affected, respectively. Then to determine the spectrum of resistance (broad or narrow) against the populations of the pathogen Pseudomonas syringae pv syringae (Pss) a collection of Pss has been obtained, which is being characterized in function of their diversity and virulence genes. Both the nature of the resistance that is present in 'Santina' and the diversity of the Pss population will determine the strategy of breeding to obtain more resistant varieties of sweet cherry to bacterial canker.

Funding: Ministerio Agricultura de Chile Proyecto Núcleo P15-16, Gobierno Regional de O'Higgins FIC-R 30474707-0; Agencia Nacional de Investigación y Desarrollo Anillo Regional ACT0190001.

3.- "Identification of defense-responsive genes in sweet cherry against Pss strains with different virulence levels"

Alan Zamorano, Laboratorio de Fitovirología, Departamento de Sanidad Vegetal, Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago, Chile

After graduating as a Biochemist at the University of Chile, my work was directed to study intracellular plant pathogens, such as viruses, viroids and phytoplasmas, focusing mainly on the molecular characterization of new species of pathogens identified in Chilean crops. Later, after completing my PhD in Agricultural Sciences, my research focused on using the information obtained from molecular characterization to optimize and improve diagnostic techniques of these pathogens to minimize their dissemination in national fields. As the development of massive sequencing techniques progressed, it happened the same with my interest in using them, originally, as a method of diagnosis and identification of new intracellular pathogens, but later this was extended to the study of the interactions between hosts and pathogens, becoming the main focus of my research. Thus, it was possible to complete the picture of the study of bacteria as scarcely studied as phytoplasmas, in this case identifying new species, new insect vectors and also determining the mechanisms of virulence of these bacteria against their hosts. Currently, I am working on research projects focused on all the mentioned topics, from optimization of diagnosis of viruses and phytopathogenic fungi, etiology of native forest diseases and identification of defense mechanisms in the process of interaction between necrotrophic bacteria with their plant hosts.

Abstract

Bacterial canker, caused by *Pseudomonas syringae* pv syringae (Pss), is the most economically important disease that affects sweet cherry trees in Chile. Its control is particularly complicated because of the ability to induce a broad spectrum of symptoms depending on the strain that infects the plant. To understand the differences observed on disease level, we proposed to challenge plants of sweet cherry, cultivar Lapins with three strains of Pss with contrasting levels of pathogenicity. Inoculations of $1x10^6$ UFC were done on the non-lignified branches of the plants. Forty days after inoculation, total RNA was extracted from the tissues located immediately after the lesion (local expression) and 20 cm farther from the lesion (systemic expression). We performed RNAseq, with 50 MM reads per sample (three replicates per sample). Raw reads were trimmed and assembled using CLC genomics workbench v24.0.1 and were later analyzed using CLC RNAseq tools for comparative gene expression analyses. Results showed that 597 genes were differentially expressed in the local response only against the hypo-virulent strain, meanwhile all the strains did not show significant differences the evaluation of the systemic response. According to its involvement in plant defense, five genes with

the highest fold-change of expression were selected for evaluation of its individual gene expression against synthetic elicitors from bacterial and fungal origin. SARD1 and TLP showed increased expression after treatment with flg22 (bacterial elicitor), meanwhile its expression decreased when treated with EIX (fungal elicitor). Further analyses are in process to identify more genes related with plant defense in sweet cherry.
Funding: This work was supported by the Proyecto Anillo O'Higgins ACT0190001 PIA-ANID

4.- "Involvement of the cell wall and its dynamic in plant defense against aphids"
María Francisca Blanco-Herrera, Centro de Biotecnología Vegetal, Facultad de Ciencias de la Vida, Universidad Andres Bello, Santiago, Chile

I obtained the degree of Doctor in Biological Sciences in 2009. I started a postdoctoral position at the Center for Plant Biotechnology at the Universidad Andrés Bello to become a regular academic in 2010. My research lines have focused on plants cellular and molecular responses, mainly to biotic stress, using the bacteria *Pseudomonas syringae* as a phytopathogen model. We have incorporated phytophagous aphids *Myzus persicae* and *Brevicoryne brassicacea* in the last seven years in their interaction with Arabidopsis. My group's work has focused on signaling pathways involved in plant responses to pathogen infections and how the pathogen manipulates the host to its advantage. In addition, some aspects of my group have been involved in studying the balance between the defense response and plant development, the structure and function of the cell wall, transcription factors, and the participation of phytohormones in the physiological process triggered by the infection/infestation of different model plants. We have been exploring the role of WRKY7, 11, and 17 transcription factors in regulating ER- chaperones genes during A. thaliana immunity. We showed that WRKY7, 11, and 17 are negative regulators of immunity through the downregulation of ER-chaperone genes. Likewise, my lab demonstrated a defensive role of the pectic polymer homogalacturonan over aphid feeding and infestation processes for the first time. Therefore, this work unveiled the answer to an old hypothesis and provides new insights into the field of aphid behavior and in the plant defense mechanism against this crop pest. In addition, he uses systems biology approaches to understand the underlying mechanisms that govern plant immunity and growth responses that ultimately lead to pathogen success and plant disease. Finally, we have been incursion in an emerging subject in the plant-pathology field, hierarchical plant defense responses against multi-attackers, and climate change, representing the starting point of my future research.

Abstract

Aphids are phloem-feeding insects that transmit viral diseases in commercial crops, a major threat to world agriculture. Aphids' feeding strategy consists of using a slender stylet that probes the host tissues using intercellular spaces as the main pathways to reach the phloem. It is thought that pectin degradation facilitates stylet penetration through the cell walls. However, host plants may take advantage of this feeding mechanism since they have evolved to sense the byproducts of pectin degradation called oligogalacturonides (OGs) and trigger a defense response. OGs are well-known damage-associated molecular patterns (DAMPs) that enhance plant resistance against pathogens. However, whether plants use this defense mechanism against aphids is unknown yet. We show that pectin and pectin-modifying enzymes are significantly altered during the plant-aphid interaction. As a result, *M. persicae* infestation induced a significant increase in total PME and PL activities, concomitant with a decrease in the methyl-esterification degree of pectin. In addition, Arabidopsis plants mutant for the PME inhibitor 13 (*pmei13*) showed increased susceptibility to both *M. persicae* and the Brassicaceae specialist aphid *Brevicoryne brassicae*. Moreover, OGs treatments in Arabidopsis increase the resistance to M. persicae infestation by reducing their offspring number and feeding performance. This enhanced resistance was related to a high accumulation of ROS and callose deposits, an increase in the transcript level of genes related to the salicylic acid pathway, such as SARD1 and PR1, and the activation of the salicylic acid (SA) signaling pathway.

Funding: This work was supported by the Fondo Nacional de Desarrollo Científico y Tecnológico [ANID-FONDECYT regular 1210320] - Programa Iniciativa Científica Milenio - ICN17_022, NCN2021_010, and ANID PIA/BASAL FB0002 to F.B-H. [ANID-FONDECYT Postdoctorado 3200902] to C.S-S.

SYMPOSIUM
"Unveiling the complex world of RNA"

Chair: Marcelo López-Lastra, Laboratorio de Virología Molecular, Instituto Milenio de Inmunología e Inmunoterapia, Departamento de Enfermedades Infecciosas e Inmunología Pediátrica, Escuela de Medicina, Pontificia, Universidad Católica de Chile.

Marcelo López-Lastra studied Biochemistry at the Universidad Austral de Chile (1994). As an undergraduate, he studied birnaviruses under the guidance of Dr. Ana Maria Sandino and Eugenio Spencer. In 1995 MLL entered the Microbiology Graduate Program, Faculty of Science, Universidad de Chile. In 1996 he was admitted to the Graduate Program, Université Claude Bernard Lyon-I, France. Working under Professors E. Spencer (Chilean Program) and Jean-Luc Darlix (French Program), MLL conducted work focused on the mechanism of translation initiation of simple retroviral mRNAs and developing retroviral vectors, graduating from both programs in 1999. He continued his research under Prof. J-L. Darlix at the ENS-Lyon, studying the mechanism of translation initiation of the SIV mRNA as an ANRS-Postdoctoral fellow. In 2001 MLL moved to Canada to continue research in Prof. Nahum Sonenberg's laboratory at McGill University as a CIHR-Postdoctoral fellow, studying translation initiation of the HIV-1 and HCV mRNAs. In 2004 he returned to Chile establishing the Laboratorio de Virología Molecular, at the Centro de Investigaciones Medicas, Escuela de Medicina, Pontificia Universidad Católica de Chile, where today he hold the position of Full Professor. Research in the MLL laboratory focuses on understanding non-canonical mechanisms of translation initiation of RNA viruses, viral antagonism of the host antiviral response, and the association between host SNPs and virus pathogenicity.

1.- "Evaluation of the impact of m6A methylation on RNA structure"
Bruno Sargueil, Université Paris Cité, CNRS, UMR 8038/CiTCoM, F-75006 Paris, France.

Bruno Sargueil is director of research for CNRS. He leads the group "structure and translation of viral RNA", in the laboratory "Therapeutic target identification and drug design", UMR 8038. He obtained his PhD at Sorbonne University, in France in 1993 working about self-splicing mobile introns. He was a postdoctoral research associate at Vermont University (USA) with Prof. J. Burke where he learnt in vitro Darwinian evolution techniques and then at Cambridge University (UK) with Prof. C.W Smith working on mammalian splicing. Since then he continued research in the RNA field mostly focused on the influence of viral RNA structure on their own translation. Most significant results include

the discovery of an original translation initiation mechanism in HIV, and insights into the molecular mechanisms involved in viral translation of various viruses such as the Human Hepatitis C Virus. His recent work focuses on collaborative multidisciplinary projects involving chemistry and computer science and aiming at better characterize and model RNA structure, dynamics and interactions.

Abstract

Many RNA functions rely on their three-dimensional structure. This includes amongst other properties interactions with the ribosome, with proteins, small molecules, and intrinsic catalytic activities. Unfortunately, RNA are poorly amenable to biophysical structure determination techniques such as X-Ray diffraction and NMR. RNA structure are therefore modeled in silico with the help of experimental data. In a multidisciplinary effort, including chemistry and computer science, we develop workflows to probe and model RNA structure and their interactions with small and macro molecules. Recent advances in epitranscriptomics have shown that chemical modification of nucleotides such as adenosine methylation, have important effects on RNA functions. These are believed to depend primarily on interactions with reader proteins that specifically recognize methylated adenosines. Little is known about the impact of methylation on RNA structure. We are currently developing chemoenzymatic tools to map epitranscriptomic modification and assess their impact on RNA structure. These methods applied to ribozymes, riboswitch or viral RNA will be presented and discussed.

Funding: This work was supported by grants from the ANR: PARNASSUS (ANR-19-CE45-0023-02) and ANR DECRYPTED (ANR-19-CE30-0021-03) and ANRS (J19P08ANRS01).

2.- "Modified RNAs as Molecular Tools for Structural and Functional Studies of aminoacyl transferases and methyltransferases enzymes"

Mélanie Etheve-Quelquejeu, Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, Université Paris Cité, Paris, France

Mélanie Etheve-Quelquejeu is professor of chemistry and the head of the Master program "Frontiers in Chemistry" at the university of Paris Cité. She leads the group "Chemistry of RNAs, Nucleosides, Peptides and Heterocycles", in the laboratory "Chimie & Biochimie, Pharmacologiques et toxicologiques", UMR 8601. She obtained her PhD at Sorbonne University, in France in 1997. She conducted postdoctoral studies at Stanford University in California with Prof. J. Collman and then at the university of Santa Barbara with Prof. B. Lipshutz. She works on the field of Chemical biology of RNAs and she developed chemical tools to explore the synthesis of cell wall of bacteria and epitranscriptomic processes. These projects involve the chemistry of nucleotides and nucleic acids, the synthesis of peptides and b-lactam derivatives and methodological developments for post-functionalization of biomolecules. She is author of more than 70 publications, including J. Am. Chem. Soc, Angewandte, NAR. She was scientific officer at the Institut de Chimie du CNRS (section 16) between 2016 and 2021.

The investigation of the role of RNAs in cellular processes is one of the most dynamic areas in Biology. In addition to their key functions in protein synthesis, new function and type of RNA are constantly discovered. To study biological processes involving RNAs, we develop synthetic methodologies to obtain stable and reactive RNAs or RNA-conjugates. The use of nucleoside and nucleotides chemistry, solid phase support synthesis, enzymatic reactions, or post-functionalization methods allowed us to obtain a large variety of modified RNA, which will be presented here. These RNA analogs were used for the study of aminoacyl transferases, a tRNA-dependent family of enzymes that catalyze an essential step in peptidoglycan synthesis of bacteria and for the study of m6A-RNA methyltransferases involved in important epigenetics events in humans, virus, or bacteria.

Funding: This work was supported by grants from the ANR: ARNTools (ANR-19-CE07-0028) and ANR SyntRNA (ANR-17-CE07-0041-02) and ANRS (J19P08ANRS01).

3.- "Mechanochemistry of the HIV-1 reverse transcriptase on an RNA substrate, one molecule at a time"

Andrés Bustamante, Instituto Milenio de Inmunología e Inmunoterapia, Laboratorio de Virología Molecular y Celular, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Santiago, Chile.

I obtained my MSc and Ph.D. degrees from the University of Chile at Santiago de Chile. During this time, I was interested in protein folding, especially in proteins with non-trivial topologies such as knotted proteins and domain-swapped dimers. Characterizing these proteins requires the ability to manipulate the topology of the unfolded state. This can't be done using traditional ensemble methods. Therefore, we used single-molecule mechanical manipulation techniques. Thus, we characterize the entropic energy cost of knot formation in the unfolded state of proteins and the kinetic steps of Domain-Swapping of the forkhead domain of FoxP1.
After my Ph.D. I decided to investigate reverse transcription. Since reverse transcriptase (RT) is key for retroviral infection, its biology and enzymology have been deeply studied. Nevertheless, there is a crucial aspect of RT function that hasn't been explored much: its behavior as a molecular motor. I'm interested in the mechano-chemical relation between the conformational changes of RT and translocation along its substrates. There is a general mechanism that could be shared between all RT. To assess this hypothesis, I'm studying the mechano-chemistry of HIV-1 RT and how Non-Nucleoside Reverse Transcriptase Inhibitors affect its mechanical behavior at the single-molecule level using high-resolution Optical Tweezers.

Abstract

HIV-1 reverse transcriptase (RT) unfolds double stranded structures by applying force to the highly structured ssRNA HIV genome during translocation and polymerization. This process is performed without the contribution of helicases, acting as a molecular motor. Even though this feature is key to the viral cycle, few studies have focused on the mechanical aspects of RT's function. In this work we developed an experimental framework to study the mechanical features of RT's RNA dependent DNA polymerization at the single-molecule level, in real time. We used a hybrid substrate formed by an ssRNA hairpin flanked by two 5000 bp dsDNA handles. Hybrids were characterized using high-resolution Optical Tweezers. Analysis of force-extension curves showed a contour length for the ssRNA of ~ 300 nm which correspond to a ~460 nucleotide (or 230 bp) hairpin. To study the polymerization reaction, DNA/RNA hybrids were held at constant force (8 pN) and incubated with 10 nM RT. During polymerization, RT unfolds the hairpin by 1 bp to insert a nucleotide. Therefore, the reaction was followed by measuring the increase of

the end-to-end distance of the whole molecular construction over time. Polymerization trajectories showed activity bursts along with inactivity periods or pauses. The calculated average pause-free velocity was ~20 bp/s. These characterizations have not been reported previously on an ssRNA substrate. Moreover, this system would allow to investigate further the mechanical features of the RT activity.

Funding: This work was supported by ANID-FODECYT (project numbers 3200460, 1190156, 1191153), ANID-FONDEQUIP (project number EQM180114) and ANID-ICM (project number ICN2021_045)

4.- "Regulation of retroviral non-canonical messenger RNA translation by RNA-binding proteins"

Marcelo López-Lastra, Laboratorio de Virología Molecular, Instituto Milenio de Inmunología e Inmunoterapia, Departamento de Enfermedades Infecciosas e Inmunología Pediátrica, Escuela de Medicina, Pontificia, Universidad Católica de Chile.

Abstract

The retroviral messenger RNAs (mRNAs) entirely resemble a cellular mRNA having a 5'cap structure, 5'untranslated region (UTR), an open reading frame (ORF), 3'UTR, and a 3'poly(A) tail. The primary transcription product interacts with the cellular RNA processing machinery and is spliced, exported to the cytoplasm, and translated. However, a proportion of the pre-mRNA subverts typical RNA processing giving rise to the full-length RNA (vRNA). In the cytoplasm, the vRNA fulfills a dual role acting as mRNA and genomic RNA. Translational control of retroviral protein synthesis is mainly exerted at the initiation step of protein synthesis. Retroviral vRNAs, can use a dual mechanism to initiate protein synthesis, a cap-dependent initiation mechanism, or via internal initiation using an internal ribosome entry site (IRES). In this study, we highlight the role of RNA-binding proteins (RBPs) that act as IRES trans-acting factors (ITAFs) and their post-translational modifications in regulating retroviral vRNA IRES-mediated translation initiation. We provide data showing that retroviral vRNA IRES-mediated translation initiation depends on ITAF recruitment and how these RBPs are post-translationally modified.

Funding: ANID though FONDECYT 1210736, and the Iniciativa Cientifica Milenio (ICM), Instituto Milenio de Inmunología e Inmunoterapia (ICN09_016/ICN 2021_045).

SYMPOSIUM
"DNA viruses and cancer: signaling pathways involved in cancer progression"

Chair: Francisco Aguayo González, Laboratory of Oncovirology, Virology Program, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile

Obtained his bachelor's degree in Biochemistry at University of Chile in 1992 and received a Ph.D. in Medical Sciences with mention in Persistent and Oncogenic viruses at Faculty of Medicine, University of Kagoshima, Japan, in 2007. His doctoral dissertation was focused on the role of high-risk human papillomavirus (HR-HPV) in extragenital tumors. Currently, he is Associate Professor at Virology Program, Institute of Biomedical Sciences (ICBM), Faculty of Medicine, University of Chile and Associate Researcher at Advanced Center for Chronic Diseases (ACCDiS). He has postdoctoral experience in Europe and U.S., working in different models of viral carcinogenesis. Since the infection with oncogenic viruses is not a sufficient condition for carcinogenesis, his research interests are signaling pathways and mechanisms related to interactions between persistent viruses and environmental factors, such as tobacco smoke. Now, he is researching the mechanisms involved in a cooperation between oncogenic viruses including human papillomavirus (HPV) and Epstein-Barr virus (EBV) and tobacco smoke components for human carcinogenesis. He serves an editor of journals in oncology, and infectious diseases.

1.- "Molecular pathways altered by early expressed proteins from Human Papillomavirus"
Marcela Lizano Soberón, Unit of Biomedical Research in Cancer, Intituto Nacional de Cancerología and Biomedical Research Institute, Universidad Nacional Autónoma de México.

Marcela Lizano obtained her Ph.D. in Biomedical Sciences at the National Autonomous University of Mexico in 1998. Currently she is principal investigator and head of the Laboratory of Epidemiology and Molecular Biology of Oncogenic Viruses at the Unit of Biomedical Research in Cancer at the National Cancer Institute of Mexico and the Biomedical Research Institute of the Universidad Nacional Autónoma de México (UNAM). The lines of research of her laboratory focus on elucidating the alteration of cell signaling pathways and cellular processes related to cancer by human papillomavirus (HPV) early expressed proteins and the identification of possible prognostic biomarkers associated to clinical outcome of patient with cervical and head and neck cancers. She also participates in epidemiological studies on HPV types and intratype variants in HPV-related cancers in the Mexican population.

Abstract
Cellular communication involves mechanisms by which cells transduce external stimuli, to control gene transcription which finally impacts in biological effects. In cancer, distinct signaling pathways including PI3K/Akt, Wnt/β-catenin, Notch and apoptosis pathways have been implicated in the deregulation of critical molecular processes that affect cell proliferation, differentiation, and death. We have found that HPV E6 and E7 oncoproteins directly alter those signaling pathways, promoting, and maintaining cancer phenotype. New interactions of HPV oncoproteins and other early expressed viral proteins, such as E1 and E2 with cellular proteins have gained attention to understand HPV-related carcinogenesis. Currently, our studies are focused on deciphering the participation of HPV early expressed proteins in the modulation of molecular pathways associated to malignant transformation, which represents a promising field for the identification of prognostic biomarkers and molecular targets for the design of therapeutic strategies.

2.- "Regulation of extracellular matrix components during human papillomaviruses mediated carcinogenesis"

Enrique Boccardo Pierulivo, Laboratory of Oncovirology, Department of Microbiology, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brazil.

Dr. Boccardo is a molecular biologist with over 25 years of experience in academia. Dr. Boccardo was graduated from the Faculty of Sciences of the University de la República, Uruguay, in 1994. He completed his MSc in Cellular and Molecular Biology in 1997 at the University de la República, Uruguay. He completed his PhD in Biochemistry in 2002 at the University of São Paulo. He has served as a scientific researcher at the Ludwig Institute for Cancer Research, São Paulo branch, during 2002-2010. Since 2010 he is a Associate Professor and Head of the Laboratory of Oncovirology in the Department of Microbiology of the Institute of Biomedical Sciences at the University of São Paulo. Dr. Boccardo is member of Latinoamerican Academy of Sciences (ACAL). In 1998 he was awarded with National Academy of Medicine of Uruguay award. Dr. Boccardo leads nationally funded research projects with a focus on the mechanisms of human papillomavirus mediated cell transformation, genome instability induction, immune evasion establisment, and in the development of epithelial organotypic cultures. Besides, he is involved in projects that aim the identification of prognosis/progression molecular markers for HPV associated pathologies. He has written and co-authored more than 60 peer reviewed publications and book chapters.

Abstract

Infection with a group of human papillomaviruses (HPV) is the etiological cause of almost the totality of cervical cancers and the great majority of anal carcinomas. Besides infection with these viruses is associated with a relevant faction of vulvar, vaginal and penile carcinomas and of an increasing fraction of head and neck tumors. Overall, almost 5% of all cancers worldwide (630,000 new cancer cases per year) are attributable to the infection with HPV: 8.6% in women and 0.8% in men. HPV are small DNA non-enveloped viruses that infect the keratinocytes of skin a mucosa HPV express two oncogenes, E6 and E7, that alter different cellular signaling pathways to warrant viral persistence and replication. As a consequence of viral oncogenes activity infected cells exhibit alterations in the regulation of cell cycle, resistance to apoptosis, immune evasion and genome instability. In fact, DNA repair machinery is essential for both HPV replication and tumor cells survival suggesting that cellular DNA repair machinery may play a dual role in HPV biology and pathogenesis. Besides, HPV infections and associated tumors are characterized by alterations in the balance between the expression and activity of extracellular matrix (ECM) components such as matrix metalloproteinases (MMP) and many of its regulators. Here, we will discuss recent data concerning the dependence of HPV-transformed cells on specific components of the cell DNA repair machinery and the role of ECM homeostasis in HPV-mediated carcinogenesis.

3.- "High-risk human papillomavirus (HR-HPV) and Epstein Barr virus (EBV) co-presence in oral cancer"

Julio César Osorio Patiño, Universidad del Valle, Cali, Colombia

Julio César Osorio Patiño is biologist (2006) and Ph.D. in Biomedical Sciences (2020) from the Universidad del Valle, Cali, Colombia. His Ph.D. dissertation was entitled "Characterization of co-infection of human papillomavirus 16 and Epstein-Barr virus in vivo and in vitro". Additionally, he is full professor at "Institución Universitaria Colegios de Colombia" from 2021. Currently, he works as a Post-Doc in the Virology Program at the Faculty of Medicine, University of Chile, on the role the Epstein-Barr virus latency in lung epithelial cells exposed to benzopyrene, using epidemiological and experimental approaches.

Abstract

Epstein-Barr virus (EBV) and high-risk human papillomaviruses (HR-HPVs) are both associated with cancers of the head and neck area. The stratified squamous surface epithelium within the head and neck region is generally permissive for HPV infection. Likewise, the oral cavity is a primary site for EBV infection and transmission. Epidemiological studies suggest that subjects with oral cancer can be infected with two or more different viruses. Indeed, exposure to both EBV and HPV is much more common than the incidence of this malignancy. HR-HPV/EBV co-presence in oral cancer would allow an oncogenic interaction between these viruses. Indeed, HR-HPV/EBV co-presence has been independently reported in oral cancer and oropharyngeal cancer specimens. We recently reported for southwest Colombia that it is possible to find the two oncoviruses in oral carcinomas as well as in benign lesions of the oral cavity. Mechanisms potentially involved in a cooperation between HPV and EBV for oral carcinogenesis are proposed.

4.- "Crosstalk between persistent viruses and tobacco smoke in carcinogenesis"

Francisco Aguayo González, Laboratory of Oncovirology, Virology Program, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile

Abstract

Epstein-Barr virus (EBV) and Human papillomavirus (HPV) are ubiquitous persistent viruses, involved in human carcinogenesis. However, the infection with these viruses is not a sufficient condition for promoting cancer. Tobacco smoke is a highly prevalent environmental factor involved as a cofactor, although the mechanisms are unclear. Indeed, virus/tobacco co-carcinogenesis involves a complex network of interactions leading to cancer initiation, promotion and progression. We have demonstrated that tobacco smoke can activate viral promoters in a dose-dependent manner, for increasing oncogene expression, in turn leading to cancer progression. In addition, PI3K/Akt signaling pathway activation is critical for tobacco smoke-dependent oncogene expression in epithelial cells. Additionally, up-regulation of superoxide dismutase 2 (SOD2) and Pirin, both involved in oxidative stress response, occurs in epithelial cells exposed to tobacco smoke components and HR-HPV oncoproteins. The interplay between persistent viral infections and environmental carcinogenic factors represents an opportunity to dissect the mechanisms involved in carcinogenesis.

SYMPOSIUM
"Yeast functional genomics"

Chair: Francisco Salinas , Universidad Austral de Chile, Valdivia, Chile

Francisco Salinas is Engineer in Molecular Biotechnology (U. de Chile) and PhD in Microbiology (U. de Santiago de Chile). He was postdoctoral researcher in the laboratory leaded by Dr. Gianni Liti (Nice, France), working on yeast population genomics. Then, he moved for a second postdoctoral experience in the laboratory leaded by Dr. Luis F. Larrondo (PUC, Chile), working on yeast optogenetics. Currently, Francisco is assistant professor and research group leader at the Institute of Biochemistry and Microbiology, Universidad Austral de Chile, Valdivia, Chile. His research group combines different synthetic biology strategies (CRISPR and optogenetics) for functional characterization of horizontally acquired genes in S. cerevisiae. In addition, his research group is also interested in the development of new optogenetic systems to control metabolic genes in yeast, by assembling light-dependent genetic circuits for transcriptional activation or repression.

1.- "The fitness landscape of telomere variation"

Gianni Liti, Université Côte d'Azur, CNRS, INSERM, IRCAN Nice, France

Gianni Liti studied biology and obtained his PhD in 2000 from the University of Perugia (Italy). In 2001, he moved to the UK (first to Leicester then to Nottingham) and worked on genome evolution, population genomics and telomere biology using the budding yeasts, S. cerevisiae and other closely related species, as model organisms. In 2011, he moved to Nice (France) as CNRS researcher where he leads a team working on yeast population genomics and complex traits analysis.

Abstract

Telomeres are ribonucleoproteins that cap chromosome-ends and impede the activation of the DNA-damage-response (DDR). Their length is maintained by telomerase using a RNA template. Telomeric sequences are conserved across the tree of life and consist of G- rich repeated units, but variation in length and sequence is present among distant taxa. Each species is likely fine-tuned for its own telomere properties, however their quantitative contribution to fitness remains largely unexplored. In the budding yeast Saccharomyces cerevisiae, the telomerase RNA template is encoded by TLC1 and carries degenerated TG1-3 repeats. Previous studies showed that a 16-bp editing of TLC1 enables to reconfigure telomeres and generate yeasts with newly synthetised human-like T2AG3 repeats, which show an intrinsic telomere dysfunction and a chronic activation of DDR. In this work, we evolved multiple lines of humanized and wild-

type yeasts to characterize the effect of telomere variation on fitness. We sequentially combined two experimental evolution paradigms: first, we evolved cells through mutation accumulation lines (MALs) to minimize selection, to investigate the cellular and genomic effects during the fitness decay driven by telomere dysfunction. Next, we submitted MALs to adaptive evolution by multiple serial transfers (STs) of large population sizes, to map mutations that counteract the fitness decline. During MALs, humanized yeasts gradually slowed their growth and shortened chronological lifespan. Whole-genome- sequencing revealed that they had increased mutation rate and genome instability, with recurrent adaptive aneuploidies on chromosome XVI. After multiple STs, most humanized lines recovered a wild-type fitness, with independent occurrence of mutations in the MRX complex, a key effector of DDR. Overall, our results show that humanized telomeres increase mutation rate and cause a severe fitness decline which is rescued by adaptive aneuploidies and the modulation of DDR.

2.- "Deciphering the molecular bases of wort adaptation in novel lager yeast hybrids"
Jennifer Molinet, Laboratorio de Genética Molecular, Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile

During my undergraduate studies and PhD degree, my research was involved in yeast genetics and biology molecular, learning from *Saccharomyces* hybrids and the molecular tools that allow us to identify them. In addition, our group-lab could work with yeast natural population, in the identification of genetic variants that underlie phenotypic differences, the validation of this genetic variants and to learn about the fermentation process. In this sense, we evaluated the effect of the allelic diversity in the TORC1 signaling pathway involved in nitrogen consumption during wine fermentation by QTL mapping on a recombinant population using four parental strains. By now, I am a postdoctoral researcher in the Molecular Genetics Laboratory of Dr. Francisco Cubillos, dedicated to understanding the genetic bases and molecular mechanisms that underlie the natural phenotypic variation among individuals, particularly in native Patagonian yeasts isolated in Chile, such as *Saccharomyces eubayanus*, and establish their potential biotechnological applications. One focus is the generation de novo *S. cerevisiae x S. eubayanus* hybrids for lager beer fermentation. In this way, we aim to respond scientific questions, such as: What is the extent of the phenotypic landscape in new hybrids? Can we emulate the fermentation performance of commercial hybrids? What are the genetic solutions in lager hybrids to long-term wort adaptation? Are the genetic solutions already established in commercial hybrids? For this, we are exploiting the genetic and phenotypic diversity in Chilean isolates from different geographic origin of *S. cerevisiae* and *S. eubayanus* strains, generating interspecific yeast hybrids and exposing to selective pressure for 200 generations to generate ethanol tolerant variants with improved fermentation capacities.

With the whole-genome sequencing of this variants, we expect to identify the molecular changes underlying an improved fermentation capacity.

Abstract

Interspecies *Saccharomyces* hybrids are possible to generate due to the absence of a pre-zygotic barrier between species and are tightly associated with domestication in industrial processes. The best-known yeast hybrid example, *Saccharomyces pastorianus* (*S. cerevisiae x S. eubayanus*), is widely used to produce lager-pilsner beer at low temperatures, which is currently the most produced alcoholic beverage worldwide. Given that the exact *S. cerevisiae* and *S. eubayanus* parental genomes of *S. pastorianus* are not available, the evolutionary history of the lager hybrid reported until now is based on the sequence analysis of other reference genomes from the parental species or other lager hybrid genomes. In this sense, the complex molecular origin and how genome plasticity under environmental stress prompted a greater fitness in lager hybrids compared to parental lineages is unknown. Thus, understanding how hybrid genomes stabilize and evolve is an essential step to determine the origin of hybrid vigor, and to elucidate how a different genome configuration can facilitate rapid adaptation to changing environments compared to parental structures. Here, we determined the genome plasticity and evolvability of *S. cerevisiae x S. eubayanus* laboratory hybrids compared to parental lines under different environmental conditions normally found in fermentation. Our results demonstrate that hybrids rapidly adapt to stressful conditions and greatly improve their fitness compared to either *S. cerevisiae or S. eubayanus* parental strains. Whole-genome sequencing demonstrated signatures of selection that explained this greater fitness. Our results demonstrate the greater genomic plasticity of hybrids compared to parental species to adapt to stressful environments.

Funding: This work was supported by ANID-FONDECYT grants 1220026 and 1180917; ANID-FONDECYT POSTDOCTORADO grant 3200545 and 3190532; ANID-Millenium Science Institute Program -ICN17_022; FIC “Transferencia Levaduras Nativas para Cerveza Artesanal”; and Fundación Ciencia & Vida.

3.- "A cohesive panel of 142 telomere-to-telomere genome assemblies unfolds the structural genetic diversity at the species-scale in Saccharomyces cerevisiae"
Gilles Fischer; Sorbonne Université, France

Gilles Fischer obtained a PhD in Genetics from the University of Nancy, France. He moved to the University of Oxford (UK) as a post-doctoral fellow where he started working on yeast genome evolution. He is now Director of Research at the CNRS and heads the Biology of Genomes team in the Laboratory of Computational and Quantitative Biology at Sorbonne University in Paris. His fields of expertise are molecular genetics and comparative genomics. His research focuses on genome dynamics through characterization of chromosomal structural variations and sequence polymorphisms at different scales, from cells to populations, and from strains and to species. His interest also includes the relationships between replication/recombination and the evolution of gene content, gene organization and variations of the nucleotide composition of genomes.

Abstract
The concept of the reference genome has outlived its purpose and should soon be replaced by the new paradigm of the pangenome Reference Assembly Panel (RAP) comprising multiple contiguous telomere-to-telomere genome assemblies that faithfully represent the genetic diversity of the species. Long-read sequencing gives access to truly complete genome sequences, including repetitive and structurally complex loci that generally remain unassembled in resequencing projects, and to phase haplotypes that usually end up collapsed in classical genome assemblies.
We generated the first S. cerevisiae Reference Assembly Panel (ScRAP) comprising 142 reference genome assemblies that deeply sample the genomic space of the species both in terms of sequence divergence, heterozygosity and ploidy but also geographic and ecological origins. Using the ScRAP, we have detected a total of more than 36,000 SVs, representing over 4,800 non-redundant events comprising insertions, deletions, contractions and duplications but also balanced rearrangements such as inversions and translocations. We analyzed breakpoints sequences and found a clear link between SV and replication origins. We also quantified the impact of SV on the gene repertoire and gene expression. We found that approximately 40% of the SVs directly impacted the sequence of protein coding genes through disruption, duplication or fusion. We resolved the fine chromosomal organization of structurally complex loci such as individual telomere lengths, large HGT regions and chromosomal scale introgression events. We reconstructed the genealogy of Ty elements and of tRNA gene families. We also discovered cases of complex aneuploidies where aneuploid chromosomes underwent inter-chromosomal translocations and/or large deletions, possibly providing an alternative adaptive route that would be inaccessible to simple aneuploidies.

4.- "The metabolic and genomic making of yeast biodiversity"

Chris Todd Hittinger, Laboratory of Genetics, DOE Great Lakes Bioenergy Research Center, Center for Genomic Science Innovation, J. F. Crow Institute for the Study of Evolution, Wisconsin Energy Institute, University of Wisconsin-Madison, Madison, WI, USA

Chris Todd Hittinger is a Professor of Genetics and the Primary Fuel Synthesis Team Lead for the DOE Great Lakes Bioenergy Research Center. His lab at the University of Wisconsin-Madison researches the genomic basis of yeast metabolic diversity, its evolution, and its applications in brewing and bioenergy. He is a Pew Scholar in the Biomedical Sciences, H. I. Romnes Faculty Fellow, and a National Science Foundation Early CAREER Development Awardee. He is a member of the Genetics Society of America, Society for Molecular Biology and Evolution, Society for the Study of Evolution, American Association for the Advancement of Science, Mycological Society of America, and represents the USA on the International Commission on Yeasts. His current tenure as Director of the J. F. Crow Institute for the Study of Evolution at UW-Madison has emphasized diversity, equity, and inclusion by founding the first standing committee to address issues and by expanding and promoting diversity among invited speakers for its Early Career Scientist Symposium. He has co-authored more than 90 publications on topics ranging from evolutionary genomics to molecular evolution to brewing to bioenergy. With co-PIs Antonis Rokas and the late Clete Kurtzman, he is the lead PI of the Y1000+ Project (http://y1000plus.org), which is sequencing and analyzing the genomes of all known budding yeast species to build a comprehensive genotype-phenotype map and to understand how yeasts have evolved their metabolic diversity. His lab has prioritized undergraduate research experiences through the Wild YEAST Program, training approximately 3 dozen students, one-third of whom have been admitted to PhD programs, including many from backgrounds underrepresented in science. As part of this discovery-driven pedagogical project and other research, he has formally described more than a dozen yeast species and is pursuing taxogenomic approaches to formally describe dozens of more species isolated by Wild YEAST Program (http://go.wisc.edu/wildyeast) trainees

Abstract

Budding yeasts of the subphylum Saccharomycotina are as genetically diverse as the entire animal or plant kingdoms. These yeasts have radiated to fill diverse niches in every continent and biome on the planet during the last 400 million years, giving rise to the model yeast *Saccharomyces cerevisiae*, the opportunistic pathogen *Candida albicans,* and more than 1000 other yeast species. One key way that they partition niche space is through their diverse carbon metabolisms. Budding yeasts have different capabilities for consuming diverse carbon sources, and the adopt distinct processing strategies that

result in different suites of carbon products. By sequencing the genomes of and collecting high-throughput metabolic data for nearly all known species, the Y1000+ Project (http://y1000plus.org) has generated a Genotype- Phenotype Map of yeast biodiversity and metabolic function. Many gene-trait correlations correspond well with mechanistic data from model systems, but several gaps and discrepancies in the map suggest novel biology. The dataset is rich in trait-trait correlations that support ecological and metabolic trait syndromes, as well as gene-gene correlations that predict pathway membership. Many traits have been gained or lost many times during evolution, whereas other traits have evolved rarely. Here, I will highlight a few examples of yeast genomic and metabolic evolution, as well as discuss prospects for filling in the budding yeast Genotype-Phenotype Map.

Funding: This work was supported by the National Science Foundation under Grant Nos. DEB- 1442148 and DEB-2110403; in part by the DOE Great Lakes Bioenergy Research Center (DOE BER Office of Science DE-SC0018409); and the USDA National Institute of Food and Agriculture (Hatch Project 1020204). CTH is an H. I. Romnes Faculty Fellow, supported by the Office of the Vice Chancellor for Research and Graduate Education with funding from the Wisconsin Alumni Research Foundation.

SYMPOSIUM
"Chaos, panic and (dis)order in protein evolution"

Chair: César A. Ramírez-Sarmiento & Pablo Galaz-Davison, Institute for Biological and Medical Engineering (IIBM), Pontificia Universidad Católica de Chile, Santiago, Chile; ANID Millennium Institute for Integrative Biology (iBio), Santiago, Chile

César A. Ramírez-Sarmiento is an Associate Professor at the Institute for Biological and Medical Engineering (IIBM) from Pontificia Universidad Católica de Chile and Adjunct Researcher at the Millennium Institute for Integrative Biology (iBio). His research group employs experimental and computational strategies to unveil the folding-function-evolution relationships of metamorphic proteins, prototypical models to understand the emergence of novel protein folds in nature, and of bacterial enzymes that hydrolyze PET, a widely used plastic that accumulates as waste in landfills and natural environments at similar rates to its production. Recently, his research has been focused on the development of open-source protocols for the local production of enzymes and reagents used in the detection of viral infections, including SARS-CoV-2.

Pablo Galaz-Davison completed the Bachelor of Biochemistry at Universidad de Chile in 2015, and a Master in Biochemistry two years later. He is soon to become a PhD in Chemical Engineering from Pontificia Universidad Católica de Chile. His research interested has been focused on understanding enzyme function, protein folding, computational biology and molecular, with several contributions in these fields. In 2018, he published an article regarding the structural features and dynamics of a newly discovered plastic-degrading enzyme. Since 2019, he became an important contributor to the understanding of the structural interconversions in metamorphic proteins, namely RfaH and KaiB, publishing several articles regarding the energetics, folding mechanism and co-evolution of these proteins. Pablo is now moving onto the study of protein-protein interactions, using the same strategies that he developed during the PhD, and new ones.

1.- "Computational approaches to study protein-protein interactions regulating the expression and function of membrane proteins"

Ariela Vergara Jaque. Center for Bioinformatics, Simulation and Modeling, Faculty of Engineering, Universidad de Talca, Talca, Chile; Millennium Nucleus of Ion Channel-Associated Diseases (MiNICAD), Santiago, Chile

Ariela Vergara Jaque received her bachelor's degree in Bioinformatics Engineering and her PhD in Applied Sciences from Universidad de Talca, Chile. Subsequently, she carried out postdoctoral research at the National Institutes of Health and Kansas State University, USA. In 2017, Dr. Vergara joined at Center for Bioinformatics, Simulation and Modeling (CBSM). She is also part of the Millennium Nucleus of Ion Channel-Associated Diseases (MiNICAD). Her research area is focused on Computational Structural Biology of membrane proteins. Dr. Vergara uses sophisticated computational methodologies to study the structure and function of membrane proteins operating as channels and transporters. Membrane proteins catalyze the passage of a broad range of solutes across lipid membranes, allowing the uptake and efflux of crucial compounds for cells. Dysfunctions of these proteins have been implicated in severe pathological conditions; therefore, membrane proteins are attractive therapeutic targets. Dr. Vergara has performed several studies to identify mechanisms that regulate the activity of membrane proteins to treat associated diseases. Her group is particularly interested in protein–protein interactions (PPIs). A critical step towards unraveling functional relationships between macromolecules is the mapping of protein-protein physical contacts, which are studied by Dr. Vergara's group employing sequence analysis, protein structure prediction, molecular docking, molecular dynamics, and free energy calculations.

Abstract

Protein-protein interactions (PPIs) play key roles in regulating expression, trafficking, and biophysical properties of ion channels. Therefore, the identification of protein networks has become an important tool to treat channel-associated diseases. Mass spectrometry (MS)- based proteomics methods are widely used to identify protein sets participating in specific biological processes. Using this approach, our group has identified a pool of PPIs modulating the function and expression of members of the Transient Receptor Potential (TRP) channel family. Particularly, for the TRPC3 channel, a broad set of associated proteins has been found, which were processed through our web platform PPI-MASS, identifying the physical association of 14-3-3η with the channel. 14-3-3 proteins have been previously implied in the surface expression and trafficking of diverse membrane proteins; therefore, a similar role is suggested for TRPC3 channels. These channels have been involved in different channelopathies, including neurological diseases, cardiovascular

disorders, and cancer. Thus, the identification and structural characterization of proteins regulating their expression might allow the design of therapeutic agents to inhibit the formation of channel-protein interactions. The work performed by our group represents an attractive approach to identify PPIs with therapeutic potential and to design therapies, based on chemical compounds and peptide formulations, to inhibit channel-protein associations.
Funding: This work was supported by FONDECYT #1220110 and #1200917.

2.- "Alpaca-derived nanobody against viral infections"
Naphak Modhiran. School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Australia; Australian Institute for Bioengineering and Nanotechnology, Brisbane, Australia

Naphak Modhiran is a Discovery Early Career Researcher Award (DECRA) Fellow and senior researcher from the School of Chemistry and Molecular Biosciences, University of Queensland, Australia. Her research focuses on antiviral and vaccine development using multi-disciplinary approaches, involving cryo-electron microscopy, rational protein design and mouse models of viral infections. Her main interest is to obtain structural information on these proteins and their interactions with host macromolecules and translate this knowledge into the rational development of therapeutic interventions such as small-molecule inhibitors, protective antibodies, and vaccine immunogens. Recently, she focused on the broad spectrum of antivirals against multiple emerging and re-emerging viruses across multiple families such as coronaviruses including SARS-CoV-2 and flaviviruses including Dengue, Zika, and Japanese Encephalitis.

Abstract
The SARS-CoV-2 Omicron variant sub-lineages spread rapidly through the world due in part to their immune-evasive properties. This has put a significant part of the population at risk for severe disease and underscores the need for anti-SARS-CoV-2 agents that are effective against novel strains for vulnerable patients. Camelid nanobodies are attractive therapeutic candidates due to their high stability, ease of large-scale production and potential for delivery via inhalation.
Here, we characterize the RBD-specific nanobody W25, which we previously isolated from an alpaca, and show superior neutralization activity towards Omicron lineage BA.1 in comparison to all other SARS-CoV-2 variants. Structure analysis of W25 in complex with the SARS-CoV-2 spike surface glycoprotein validate shows W25 engages with a novel RBD epitope not covered by any of the antibodies previously approved for emergency use.

Furthermore, we show that W25 also binds the spike protein from the emerging, more infectious Omicron BA.2 lineage with picomolar affinity. In vivo evaluation of W25 prophylactic and therapeutic treatments across multiple SARS-CoV-2 variant infection models, together with W25 biodistribution analysis in mice, demonstrates favorable pre-clinical properties.

Together, these data endorse prioritization of W25 for further clinical development. Further, we generate and characterized nanobodies against other potential pandemic virus.

Funding: This work was supported by NHMRC MRFF Coronavirus Research Response grant APP1202445 to D.W., K.C., P.R.Y and T.M. Cryo-EM equipment at CMM is supported by ARC The Linkage Infrastructure, Equipment and Facilities (LIEF) scheme. N.M. was supported by UQ Research Stimulus. D. S. was supported by the German Research Foundation (DFG) Emmy Noether Programme (SCHW1851/1-1) and by an EMBO Advanced grant (aALTF-1650). The Chilean platform for the generation and characterization of Camelid Nanobodies to A.R.F is funded by FONDECYT No. 1200427; the regional Council of the “Los Rios region” projects FICR19-20, FICR21-01; FICR20-49 to R.J; the Bio & Medical Technology Development Program of the National Research Foundation (NRF) of the Korean government (MSIT) (NRF-2020M3A9H5112394); the ANID-MPG MPG190011 and ANID-STINT CS2018-7952 grants; and the EU-LAC T010047

3.- "Metamorphic proteins don't just wiggle wiggle, they (re)fold"

César A. Ramírez-Sarmiento. Institute for Biological and Medical Engineering (IIBM), Pontificia Universidad Católica de Chile, Santiago, Chile; ANID Millennium Institute for Integrative Biology (iBio), Santiago, Chile

Abstract

Metamorphic proteins constitute unexpected paradigms of the protein folding problem, as their sequences encode two alternative folds, which reversibly interconvert within biologically relevant timescales to trigger different cellular responses. Once considered protein oddities, recent experimental discoveries and bioinformatic analyses argue that they might be widespread.

Given that their fold-switch is directly involved in the regulation of their biological function, there is great interest in understanding the mechanisms of their structural interconversion. Here, we describe our experimental and computational efforts to characterize the structural interconversion of metamorphic proteins, using the specialized bacterial NusG paralog RfaH as an example. RfaH undergoes an all-α to all-β fold-switch of a whole protein domain to bind to RNA polymerase and activate the expression of virulence and conjugation genes in bacterial pathogens, constituting the quintessential example of a metamorphic protein. By combining structural biology, molecular dynamics, and hydrogen- deuterium exchange mass spectrometry, a consistent structural interconversion pathway is conceived, in which at least two intermediates enable RfaH to switch back and forth between its native states. Moreover, these methods enable to elucidate the effects of RfaH binding to RNA polymerase, largely inaccessible for most structural biology methods.

The experimental insights that enabled the elucidation of RfaH fold-switch can be further exploited in current state-of-the-art protein structure prediction methods to distinguish between metamorphic and non-metamorphic homologs, constituting one of the many ongoing efforts to find signatures and general properties to ultimately describe the protein metamorphome.

Funding: FONDECYT 1201684, ANID Millennium Science Initiative Program ICN17_022

4.- "Conformational sampling with AlphaFold2 by phylogenetic amnesia"

Matías R. Machado. Protein Engineering Unit, Institut Pasteur de Montevideo, Montevideo, Uruguay.

My interest for Computational Biophysics started in 2003, as a fellow in the Laboratory of Theoretical and Computational Chemistry at the School of Science from Universidad de la República, Uruguay. In 2007, I obtained a bachelor's degree in Biochemistry at the same University. Then, in 2012, I got a PhD in Biology working in the Biomolecular Simulation Laboratory at Institut Pasteur de Montevideo. Today I work as a researcher with more than 17 years of experience in the field of Molecular Modeling and Simulation, with a solid background in bioinformatics and biochemistry. My scientific focus is the understanding of biological processes from a molecular and structural point of view. My main topic of interest is the application of computational techniques to unravel the secrets of life. In that sense, my main scientific contributions are devoted to developing computational tools and molecular models to study biomolecules at atomic scale. My vision is to contribute to the society and the scientific community by providing useful knowledge and products, which can be used despite the limitations in resources. I am particularly concerned about gender and social inequalities; thus, I am an active promoter of activities to bridge the different gaps in Uruguay and South America.

Abstract

AlphaFold2 emerged as a game-changing algorithm based on artificial intelligent for protein structure prediction. Despite the success, several aspects remain elusive on how the trained neural net of AlphaFold2 can achieve such accurate predictions. Fresh ideas point to a learned highly accurate biophysical energy function (Roney and Ovchinnikov, 2022). In that scenario, the input information from a multiple sequence alignment (MSA) or template serves as a bias to restrict the conformational space during the search for a minima in the energy surface. Indeed, recent work shows how to sample alternative protein conformations with AlphaFold2 by tuning the MSA depth (del Alamo et al., 2022). In the present work, we explore this hypothesis by studying metamorphic proteins, in which the same sequence encodes two coexisting native conformations, challenging AlphaFold2 capabilities. Assuming that evolution left traces of the different native states, we propose a new method called 'phylogenetic amnesia' to systematically explore the conformational space with AlphaFold2.

Funding: M.R.M belongs to the SNI program of ANII. This work was partially funded by FOCEM (MERCOSUR Structural Convergence Fund), COF 03/11, FONDECYT 1201684 and ANID Millennium Science Initiative Program ICN17_022.

5.- "FrustraEvo: Assessing protein families divergence in the light of sequence and energetic constraints"

María I. Freiberger. Protein Physiology Lab, Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires-CONICET-IQUIBICEN, Buenos Aires, Argentina

In 2017 I completed my Bsc/MSc in Bioinformatics with the defense of my Master thesis focused on the analysis of local frustration in enzymes at the University of Entre Rios (Argentina) directed by Dr. Gonzalo Parra and Dr. Diego U. Ferreiro. In 2018 I started my PhD in Biological Chemistry at University of Buenos Aires (Argentina) under the supervision of Dr. Diego U. Ferreiro. My PhD research topic is focused on the study of protein folding and evolution. More specifically, I study the relationships between sequences, structures, dynamics, and function of proteins in the context of their evolutionary history. During my PhD I collaborated with different laboratories in different countries (USA, Italy, Germany, Barcelona, and Chile) in projects related to the study of protein folding, function and evolution using frustration. I was also involved in the development of bioinformatics tools for the analysis of energetic patterns, such as FrustraR, FrustraPoket, EvoFrustra. Finally, I worked as a collaborator in the RSG-Argentina from 2015 to 2021.

Abstract

Natural proteins fold by minimizing the energetics of those interactions that are present in their native states (Bryngelson and Wolynes, 1987). Although, not all interactions that are present in the native state can be energetically optimized. In typical globular proteins, 10-15% of the total interactions in their native states are in conflict with their local environment (Ferreiro et al., 2007). These conflicting, frustrated, signals have been linked with different functional aspects such as protein-protein interactions, allosterism (Ferreiro et al., 2011) and catalytic activity (Freiberger et al., 2019).

We have developed FrustraEvo, a tool that measures local frustration conservation patterns within protein families. We show, in the context of different protein families, that we can detect residues that are important either to the protein's stability or to different functional requirements.

FrustraEvo uses FrustratometeR algorithm (Rausch et al., 2021) to calculate local frustration in protein structures and apply information content concepts to calculate frustration conservation at the multiple sequence alignments (MSAs) columns of the different protein families. MSAs were generated with ClustalW and manually curated. We retrieved experimental protein structures when available and generated AlphaFold2 structural models when not.

FrustraEvo is a tool that measures local frustration conservation patterns within protein families as a proxy to define residues that are important either for stability or function and relate them to their sequence variability signatures. We additionally compare homologous protein families and show how specific residues have substituted their amino acid identities with direct impact in their energetic signatures while others remain invariant.

Funding: This work was supported by the Consejo de Investigaciones Cientificas y Tecnicas (CONICET), the Agencia Nacional de Promocion Cientifica y Tecnologica and Barcelona Supercomputing Center.

SYMPOSIUM
"New insights in non-coding RNAs in prokaryotic and eukaryotic cells"

Chair: Valentina Parra, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile.
Mauricio Latorre, Universidad de O'Higgins, Rancagua, Chile

Valentina Parra, Ph.D, is Associate Professor of the Department of Biochemistry and Molecular Biology of the Faculty of Chemical and Pharmaceutical Sciences of the University of Chile. Currently, she is also a Research Associate in the Advanced Center for Chronic Diseases (ACCDiS) and Scientific Advisor to PAR EXPLORA North Metropolitan Region, both also located at the University of Chile. Valentina returned to Chile in 2015 after completing her postdoctoral training at the University of Texas Southwestern Medical Center in Dallas. Professionally, she has dedicated herself to the study of mitochondrial morphology and metabolism and the molecular mechanisms involved in the development and progression of cardiovascular diseases. Currently, and as an independent researcher, she has focused on understanding the fundamental role of the mitochondria in both physiological and pathological cell differentiation processes, and recently the role of lnRNA in their regulation. During her career, she has been recognized by several national and international organizations, including the Chilean Society of Biochemistry, UNESCO, the American Heart Association, the NHLBI Progenitor Cell Biology Consortium (PCBC / NIH), and lately, the Chilean Academy of Sciences, which recognized her as the best young female scientist in 2019 and as a member of the Frontier Science group in 2022 and, Madeco by Nexans, from the Luksic Group, which recognized her as an inspiring woman in the 2019-2021 cycle.

Mauricio Latorre is Molecular Biotechnology Engineering from Universidad de Chile (Santiago, Chile) and PhD in Science, mention molecular, cell biology, and neuroscience at the same institution. His professional career is linked to areas of systems biology and biotechnology, mainly focused on the construction and use of bacterial models capable of describing resistance to heavy metals, pathogenesis, and biomining processes. He is working as an assistant professor at the Institute of Engineering Sciences of the Universidad de O'Higgins and INTA, associate researcher at the Center for Genome Regulation and Center for Mathematical Modeling, and international collaborator of the department of "Infection Diseases" of the University of Texas (USA) and the CNRS-Dyliss group of the Université de Rennes (France). Currently, he is the director of SYSTEMIX; an interdisciplinary center focused on studying microbial communities from mining tailings with applications in biotechnology.

1.- "Computational tools for identifying and characterizing non-coding RNAs in prokaryotic and eukaryotic organisms"

Raúl Arias-Carrasco, Programa Institucional de Fomento a la Investigación, Desarrollo e Innovación (PIDi), Universidad Tecnológica Metropolitana, Santiago, Chile

Raúl Arias-Carrasco is a Bioinformatic Engineer from Universidad de Talca (Talca, Chile) and PhD in Integrative Genomics from the Universidad Mayor (Santiago, Chile). His PhD Thesis was versed in the study of Non-coding RNAs in archaeal species living at different temperatures. Currently, he is a researcher in the Institutional Program for the Promotion of Research, Development, and Innovation (PIDi) at the Universidad Tecnológica Metropolitana in Chile and Associate Investigator in SYSTEMIX (Systems Biology Center for the study of extremophile communities from mining tailings). Currently, his line of research lies in the use and development of computational tools for studying non-coding RNA functions, both in prokaryotes and in human diseases.

Abstract

Non-coding RNAs (ncRNAs) are present in all domains of life, playing a critical role in fine-tuning regulation of important biological processes and participating in the fundamental functional core of every biological system. This type of RNA molecule is classified in a wide range of classes and families. Their important biological role led some research groups to focus on developing new methodologies for identifying and predicting ncRNAs on nucleotide sequences. Therefore, several bioinformatics approaches have been developed and implemented to identify and predict novel ncRNAs using sequence alignments, secondary structure inference strategies and machine learning approaches. Here, we present a complete overview and applications of bioinformatics tools developed by our group to identify and characterize non-coding RNAs in any biological sequence. In this talk, we will present these tools, as well as some applications in characterizing the ncRNA repertoires in prokaryotic and eukaryotic data, including genomes, transcriptomes and metagenomes. We will focus especially on the characterization of extremophile organisms, including dozens of Archaea genomes and their association with high-temperature adaptability, as well as metagenomic sequencing data obtained from the Cauquenes tailings (SYSTEMIX), located in the O'Higgins Region (Chile).

2.- "Non-coding RNA global transcriptional regulatory network activated by iron in Enterococcus faecalis"

Victor Aliaga-Tobar, Bioengineering Laboratory, Institute of Engineering Sciences, Universidad de O'Higgins, Rancagua, Chile

Victor Aliaga Tobar is a Biotechnologist from Universidad Iberoamericana de Ciencias y Tecnología (Santiago, Chile) and PhD in Integrative Genomics from the Universidad Mayor (Santiago, Chile). Currently he has a Postdoctoral position at O'Higgins University where is studying different transcriptional regulatory mechanism included ncRNA, methylations and transcription factors on several bacterial species. In addition, he has developed several algorithms to identify and characterize small RNA families.

Abstract

Systems biology has emerged as a powerful descriptive, predictive, and integrative discipline that allows for the study of complex systems to investigate biological phenomena. For the last ten years, our group has worked with the pathogenic bacteria Enterococcus faecalis and its relationship with metals using a systems-biology approach. Taking advantage of our extensive knowledge of this organism, the present seminar will show the strategy to construct and final model of an ncRNA global transcriptional regulatory network activated by iron in E. faecalis. The combination and integration of the total set of cis-sRNA and trans-sRNA regulators differentially expressed under iron deficit and excess into the genome of E. faecalis, will represent the ncRNA global transcriptional regulatory network activated by the metal. Topological analysis was performed to identify conserved structures of ncRNA controlling the expression of several transcriptional regulatory factors, denoting a complex structure between two gene regulatory mechanisms, revealing a completely new perspective on transcriptional regulation, ncRNA and metals.

3.- "Studying the non-coding transcriptome of endocrine pancreatic cells from zebrafish"

Estefanía Tarifeño-Saldivia, Gene Expression and Regulation laboratory (GEaRLab), Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Chile

Estefanía Tarifeño-Saldivia is a Biologist from the University of Concepción and PhD from the University of Liège (Belgium). In her undergraduate studies, she was passionate about the study of the control of food intake seen from a neurobiological perspective. Then, during her doctorate, she was trained in "OMICS" sciences and bioinformatics applying them to the study of the transcriptomic fingerprint that defines and differentiates each pancreatic cell. In both of her post-doctoral stays (UCL, San Francisco and Universidad de Concepción), she studied the role of non-coding RNA associated with various biological processes. Currently, her laboratory is focused on identifying the effects of obesity on neurons that regulate food intake. In this regard, her research is based on genome-wide studies that evaluate transcriptomic and epigenomic dynamics that allows to understand phenomena typically observed in obese individuals such as hyperphagia and rebound.

Abstract

Long noncoding RNAs (LncRNAs) are functional RNA molecules involved in diverse biological processes such as organogenesis, defining cell identity, and susceptibility to developing diseases. Indeed, most of the diabetes susceptibility loci are mapped to non-coding regions of the human genome. Then, studying the function of non-coding RNA in pancreatic cell biology is crucial for understanding diabetes pathogenesis. Currently, just a few studies have indicated that lncRNA plays a role in controlling b-cell proliferation during development, b-cell mass, and b-cell identity. However, as it has been postulated, diabetes pathogenesis not only involves b-cells. The pancreas of zebrafish, at cellular composition and structural level, resembles its mammalian counterparts. Transparent embryos, several transgenic strains available, and conditional ablation allow the study of pancreatic development and function. In the present study, we explored the non-coding transcriptome of the main endocrine and exocrine pancreatic cell types from adult zebrafish. By RNA-seq and de-novo assembly, we predicted the non-coding fingerprint of beta, alpha, delta, acinar, and ductal pancreatic cells. These results are the starting point for characterizing the role of lncRNAs in pancreatic development and pancreatic pathogenesis such as diabetes.

4.- "RNA at the Epicenter of Genetic Information: from epigenetic to epitranscriptomic regulation in development and cancer"

Paulo P. Amaral, Insper Institute of Education and Research, Brazil

Paulo P. Amaral graduated in Biological Sciences at the University of Brasília, he has a Master in Biochemistry from the University of São Paulo and a PhD in Molecular Genetics from the University of Queensland (Institute for Molecular Bioscience). He was a Cambridge University Research Fellow at the CRUK/Wellcome Trust Gurdon Institute and the Milner Therapeutics Institute, with funding from a Royal Society and British Academy Newton International Fellowship, a Corpus Christi College Research Fellowship and a Borysiewicz Biomedical Sciences Fellowship. He has also worked in Industry for Pentail Enzymes (now JĀNA BIO LTD) as a co-founder and in STORM Therapeutics Limited as a researcher. He has been a visiting professor at several universities, including Campus Bio-Medico University of Rome (Unicampus) and Humanitas University – Medical School of Milan, Italy, as well as Karolinska Institutet and Uppsala University, Sweden. His research has covered several mechanisms involved in the control of genome activity during embryonic development, in stem cells and in diseases, with a special focus on regulatory networks involving non-coding RNAs ("noncoding RNAs") and their covalent modifications ("RNA modifications"). Currently, his research emphasis at Insper focuses on the intersection of molecular biology and bioengineering and the development of analytical tools and techniques for the study of biomolecules.

Abstract

The mammalian genome is transcribed into several thousand long noncoding RNAs (lncRNAs) whose properties and functions are currently being unravelled. My colleagues and I have used genome-wide and functional strategies to study their biological roles, in particular in embryonic development and cancer. Using RNA-seq to analyse expression in human and mouse tissues and cell lines, we identified over 1,000 spliced 'positionally conserved' lncRNAs in syntenic locations. This analysis identifies a new lncRNA subgroup, tapRNAs (topological anchor point RNAs), whose promoters overlap chromatin loop anchor points and contain CTCF sites. TapRNAs are associated predominantly with developmental genes with which they share common features: (a) expressed in the same tissues, (b) able to regulate each others' expression, (c) and are similarly misregulated in cancers and (d) influence differentiation of embryonic stem cells and metastatic characteristics of cancer cells in vitro. Experimental and computational evidence shows a functional association of individual tapRNA loci and chromatin organization. We have also analysed the repertoire of RNA post-transcriptional modifications (PTMs) in lncRNAs and mRNAs, using Nanocompore Direct RNA-seq and a robust statistical framework to

evaluate the presence of different PTMs. To do so, we compared native RNAs isolated from biological samples of interest, in particular of leukaemia cells, against a range of non-modified RNA controls. We applied our method to map known and novel modification sites also in conserved polyA- noncoding RNAs. Altogether, the work presented supports widespread roles of RNAs and their modifications in the control of gene expression in developmental and cancer models.

NEW MEMBERS SESSION

"Mitochondrial Ca2+ overload in the neurodegeneration associated with early-onset familial Alzheimer's disease"

Marioly Müller, Departamento de Tecnología Médica, Facultad de Medicina, Universidad de Chile

Medical Technologist, MSc in Cell Biology, and PhD in Biomedical Sciences from the Universidad de Chile. Currently, Assistant Professor at the Departamento de Tecnología Médica, Facultad de Medicina, Universidad de Chile. Dr. Müller has been working in science since her undergraduate thesis on Ca2+-dependent transduction pathways in skeletal muscle cells and later during her graduate formation. Dr. Müller conducted her PhD research at the University of Pennsylvania on calcium dysregulation in Alzheimer's disease (AD). This work focused on exploring the enhanced CREB phosphorylation mediated by familial AD mutant presenilin one associated exaggerated Ca2+ signaling. Dr. Müller has contributed to significant publications in the Alzheimer's field, cellular bioenergetics, and autophagy with relevance for neurodegenerative diseases and cancer. She is currently working on intracellular Ca2+ signaling, mainly linked to mitochondrial functions and bioenergetics in different models.

Abstract

Familial Alzheimer's disease (FAD) is characterized by mutations in presenilin-1 (PS1) gene affecting intracellular Ca^{2+} homeostasis. An imbalance between mitochondrial Ca^{2+} uptake and removal leads to the opening of the permeability transition pore, causing the loss of mitochondrial membrane potential ($\Delta\Psi m$), uncoupling of the respiratory chain, and a drop in ATP. Accumulation of mitochondrial free Ca^{2+} causes a toxic mitochondrial Ca^{2+} overload, inducing an apoptotic process contributing to the disease pathogenesis.

Purpose: We investigated the role of the FAD-mutant PS1-M146L disruptions of intracellular Ca^{2+} homeostasis on mitochondrial function using in vitro cell models.

Methods: We used SH-SY5Y cells transfected with mutant PS1-M146L and fibroblast from FAD, sporadic AD (SAD), and control patients. We performed simultaneous measurements of $[Ca^{2+}]m$ uptake and $\Delta\Psi m$ in permeabilized cells to measure MCU-mediated Ca2+ uptake. Live-cell imaging experiments were performed to estimate qualitatively and quantitatively $[Ca^{2+}]m$, using Ca^{2+} indicators.

Results: Mutant PS1 cells were subjected to elevated mitochondrial Ca^{2+} levels, but no significant differences in mitochondrial Ca^{2+} uptake through MCU or $\Delta\Psi m$ were found. Basal levels of $[Ca^{2+}]c$ and $[Ca^{2+}]m$ were significantly higher in mutant PS1 SH-SY5Y cells and fibroblasts from FAD and SAD patients. Excess Ca^{2+} levels in PS1-M146L cells triggered a sensitivity to successive Ca^{2+} challenges, causing a loss of $\Delta\Psi m$.

We found a decreased expression of NCLX in mutant PS1 cells, suggesting an impaired mitochondrial Ca^{2+} efflux.
Conclusions: These features may explain increased vulnerability and eventual death due to progressive mitochondrial dysfunction, promoting a pathological cycle essential to the disease progression of FAD.
Funding: This work was supported by the University of Chile and the University of Pennsylvania

"Epigenetics effects of obesity in the neurons that regulate feeding behavior"

Estefanía Tarifeño-Saldivia, Gene Expression and Regulation laboratory (GEaRLab), Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Chile.

I am a biologist (Universidad de Concepción, Chile), PhD in science (Université de Liège, Belgium) and since 2018 I am Assistant Professor at the University of Concepción. My academic training has provided me with tools that are currently integrated into my research. During my undergrad, I feel passionate about the study of feeding control from a neurobiological point of view. Later, during my Ph.D., I was trained in the OMICs and bioinformatics field which I applied to understand the transcriptomic fingerprint defining and differentiating endocrine pancreatic cell types. During my two post- doctoral positions, I studied the role of non- coding RNAs associated with diverse biological processes. Currently, one of my research lines is focused on studying the effects of a long-term high-fat diet on the methylome of FACS-purified POMC neurons to identify gene modulation induced by methylation dynamics. These results will open new research lines regarding the functional role of these genes in rebound weight gain and the discovery of alternative therapies. Such kind of research is of fundamental interest to our country, as Chile is becoming an obese population.

Abstract

Obesity is a chronic medical condition characterized by increased body weight and excess body fat. In Chile, 74% of the adult population suffers from overweight, and 34,4% of obesity. This metabolic condition produces systemic effects among which the neuronal circuit regulating feeding behavior is dramatically disrupted. Indeed, it has been postulated that macronutrients present in obesogenic diets (such as saturated fatty acids) induce specific effects in the hypothalamic POMC neurons such as leptin resistance, dramatic transcriptomic modulation, and loss of these neurons, leading to failure to control hyperphagia in obese mice. Long-lasting effects might be supported by epigenetic modifications - induced by obesity - that maintain gene dysregulation even after normal weight has been achieved. It has been demonstrated that obesity induces DNA hypermethylation on the Pomc promoter of POMC neurons disrupting the normal leptin signaling, but genome-wide effects have not been yet evaluated which might explain the dramatic transcriptome modulation documented for these neurons. In this work, we studied the effects of a long-term high-fat diet on the methylome of FACS-purified POMC neurons to identify gene modulation induced by methylation dynamics. These results will open new research lines regarding the functional role of these genes in rebound weight gain and the discovery of alternative therapies. Such kind of research is of fundamental interest to our country, as Chile is becoming an obese population.

Funding: This work has been funded by Fondecyt Iniciación N°11190401 (ANID/FONDECYT), VRID-Iniciación 218.037.024-INI (Universidad de Concepción)

SYSTEMIX: Systems biology center for the study of extremophile communities from mining tailings.

Mauricio Latorre, PhD. Systems biology center for the study of extremophile communities from mining tailings (SYSTEMIX), O'Higgins University, Rancagua, Chile. Laboratory of Bioinformatics and Gene Expression, INTA, University of Chile, Santiago, Chile. Bioengineering Laboratory; Institute of Engineering Sciences; O'Higgins University, Rancagua, Chile. mauricio.latorre@uoh.cl

Mauricio Latorre is Molecular Biotechnology Engineering from Universidad de Chile (Santiago, Chile) and PhD in Science, mention molecular, cell biology, and neuroscience at the same institution. His professional career is linked to areas of systems biology and biotechnology, mainly focused on the construction and use of bacterial models capable of describing resistance to heavy metals, pathogenesis, and biomining processes. He is working as an assistant professor at the Institute of Engineering Sciences of the Universidad de OHiggins and INTA, associate researcher at the Center for Genome Regulation and Center for Mathematical Modeling, and international collaborator of the department of "Infection Diseases" of the University of Texas (USA) and the CNRS-Dyliss group of the Université de Rennes (France). Currently, he is the director of SYSTEMIX; an interdisciplinary center focused on studying microbial communities from mining tailings with applications in biotechnology.

Abstract

The study of mining tailings has become a priority in Chile. To date, little is known about the structure and potential of the microorganism communities that inhabit the mining environments, relevant to both the biological and environmental scenarios, and also, for commercial interests. The Cauquenes tailings located in the O'Higgins Region, is the oldest and largest copper tailings, reservoir to date of the material deposited by El Teniente. Identifying and characterizing communities of extremophile microorganisms that inhabit the Cauquenes tailings will provide us with valuable information on the structure of the communities and how they have been maintained or changed over time.

In this context, Systems Biology has emerged in the last decade as the best alternative for providing a better understanding of complex biological systems beyond the molecular-level scale or singular species. Based on our experience working in this field, we will present different models of biological networks related to resistance to metals in pathogenic bacteria, biomining species, and extremophile communities, a knowledge that allowed us to lay the foundations for the creation of the SYSTEMIX, the first systems biology center for the study of bacterial communities that inhabit mining tailings.

We will seek to generate a database of extremophile species representative of the history of the Cauquenes tailings, focusing the studies on bacterial conservation, biological

networks, metabolism, and mainly biotechnology applications. The results of this study will provide, for the first time, characterization and temporal stability report of the structure of the extremophile communities present in these tailings.

Funding: ANID FONDECYT 1190742 (ML), ANID BASAL FB210005 (ML), ANID MILENIO ICN2021_044 (ML), ANID ANILLO ACT210004 (ML) and ACT20210044 (ML), Beca ANID 21211367 (GG) and 21220593 (JO).

““Evolution of enzyme regulation and the mechanistic drift hypothesis: The case of the activation by AMP in archaeal ADP-dependent Sugar Kinases”

Gabriel Vallejos-Baccelliere, Laboratorio de Bioquímica y Biología Molecular, Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile
Sponsor: Victoria Guixé

My scientific interests are within the field of the physical chemistry of enzymes. In the context of my Ph.D. thesis, I studied the kinetic mechanisms of enzyme regulation in the case of the archaeal ADP-dependent sugar kinases. This work has an evolutive background, intending to characterize the evolutive trajectory of the allosteric regulation in this enzyme family. Since then, my research has primarily consisted in addressing complex enzymatic phenomena characterizing their mechanisms using stationary-state kinetics, transient kinetic and thermodynamic methodologies, and applying adequate mathematical tools and models for each case. Those phenomena involve, for example, enzymes capable of catalyzing successive reactions and the effect of unspecific factors, like ionic strength, in enzyme catalysis, which are the subjects of my ongoing postdoctoral project. Parallelly, I have devoted myself to research and teaching in the field of philosophy of science, specifically, the philosophy of experimental biology and biochemistry. My current lines of research in this area include the epistemological problems that emerge from utilizing machine learning to predict macromolecular structures and from the use of artificial laboratory models to obtain knowledge about real biological systems.

Abstract
The evolution of enzyme regulation at the mechanistic level is a field that has only recently been addressed in detail. In this work, we assess this phenomenon using the archaeal ADP-dependent sugar kinase family as a case study. It is generally accepted that allosterism is almost absent in archaeal enzymes, and instead, metabolic regulation is exerted mainly at the transcriptional level. However, some examples have been reported in the last years, which include the work of our group on the bifunctional ADP-dependent glucokinase/phosphofructokinase from Methanococcus maripaludis (MmPFK/GK), which is activated by its reaction product AMP. This enzyme carried out the phosphorylation of glucose (GK) and fructose-6-P (PFK) at the same active site, and both activities are activated by AMP. To understand this phenomenon, we performed a comprehensive kinetic characterization, including the determination of the kinetic and AMP activation mechanisms of MmPFK/GK. This enzyme has an ordered sequential mechanism where MgADP is the first substrate to bind to the enzyme, and AMP is the last product to leave. Activation by AMP of both activities occurs by an increase in the affinity for the sugar substrate, with this effect being higher in the GK than in the PFK activity. Interestingly, AMP triggers substrate inhibition by increasing the sugar affinity of non-productive enzymatic forms. By studying the AMP activation in extant homologous enzymes as well as in the resu-

rrected ancestors of this family, we determined that AMP activation is an ancestral trait correlated with bifunctionality, which is lost during the evolutionary trajectory toward specific GK or PFK enzymes. Interestingly, although activation by AMP is a conserved trait, its underlying kinetic mechanism varies through evolution. This mechanistic drift has interesting consequences not only for our understanding of the evolution of allosterism, but also has broad theoretical and philosophical implications for molecular evolution in general.

Funding: Fondecyt 3210758

ORAL SESSION

"*In silico* and *in vitro* determination of the estrogenic metabolic pathways that controls the hPASMC pulmonary hypertensive pathologic phenotype"

Emanuel Guajardo-Correa[1], Juan Francisco Silva-Agüero[1], Gerardo García Rivas[2], Mario Chiong Lay[1] , Mauricio Latorre Mora[3,4,5], Valentina Parra Ortiz[1,5,6]

[1]Department of Biochemistry and Molecular Biology and Advanced Center for Chronic Diseases (ACCDiS), Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile.
[2]Tecnológico de Monterrey, Biomedicine Research Center and The Institute for Obesity Research, Zambrano Hellion Hospital, Monterrey, México.
[3]Bioengineering Laboratory; Institute of Engineering Sciences; O'Higgins University, Rancagua, Chile.
[4]Laboratory of Bioinformatics and Gene Expression, INTA, University of Chile, Santiago, Chile.
[5]Systems biology center for the study of extremophile communities from mining tailings (SYSTEMIX), O'Higgins University, Rancagua, Chile.
6Network for the Study of High-lethality Cardiopulmonary Diseases (REECPAL), University of Chile, Santiago, Chile.

Idiopathic pulmonary arterial hypertension (iPAH) is a chronic and incurable disease. Affects mainly women, being its biggest risk factor elevated estrogen (E2) plasma levels. It is unknown how E2 and its metabolism controls the cancer-like reprogramming (higher proliferation and a decreased mitochondrial oxidative metabolism) in pulmonary artery smooth muscle cells (hPASMC), the main iPAH-related cell type. Using a systems biology approach, we build a human genome-scale transcriptional regulatory network (TRN) to identify novel TF-gene interactions and metabolic pathways (MP) affected in iPAH. This MP-integrated TRN was build using public iPAH patient's gene expression and gene-metabolic databases. Parallel, we also evaluated whether E2 or its metabolite, 4-Methoxyestradiol (4-ME), modulates the cancer-like reprogramming and their related receptors in hPASMC. Cell cycle was studied using propidium iodide and flow cytometry. Mitochondrial potential (ΔΨm) and oxygen consumption rate (OCR) were measured using an OROBOROS Oxygra

ph-2k. Finally, the estrogen receptor activity was evaluated using the estrogen receptor (ER) inhibitors Fulvestrant or G36 (10 μM). The MP-integrated TRN shows an estrogenic master pathway in iPAH due to estrogen receptor alpha (ESR1)-mediated CYP1B1 upregulation of estrogen and steroids metabolism. E2 100 nM and 4-ME 5 nM (48 h) increa-

ses the proliferative G2/M phase. E2 upregulates OCR and downregulates ΔΨm, while 4-ME downregulates ΔΨm. Using the ER-inhibitors, we determined that the estrogenic proliferative effect is carried out by different receptors: ESR/E2 or GPER/4-ME, respectively. To our knowledge, this is the first work showing that the phenotypic metabolic reprogramming in iPAH is controlled by different estrogenic pathways.

Funding: This work was supported by grants 1190743 (VP) and 15090007 (ML) FONDAP 15120011 (MC, VP) and 15090007 (ML), U-Redes G_2018-35 (VP), CRP-ICGEB CHL18-04 (VP, ML) and Beca Doctorado Nacional ANID21191519 (EG-C).

"HDAC6 inhibition decreases STAT3 hyperactivation in colorectal cancer cells"

Constanza Mardones[1], M. Estrella Armijo[1], Fernando Rivas-Valdes[1], Matias Hepp[1]

[1]Laboratorio de Investigación en Ciencias Biomédica, Departamento de Ciencias Básicas y morfología, Facultad de Medicina, Universidad Católica de la Santísima Concepción, Concepción, Chile

Colorectal cancer (CRC) is one of the pathologies with the highest mortality rate in the world and even though there are different treatments, such as immunotherapy, a strategy that is directed at immune control points, but it is not totally effective. Histone deacetylase 6 (HDAC6), is involved in several critical functions related to cancer, through the modulation of the activity of transcription factors such as STAT3, which has been described as involved in the regulation of proto-oncogenes and anti-apoptotic proteins involved in different cancer, such as CRC or breast cancer. There is little information on the regulation of HDAC6 over STAT3 in CRC cells. Therefore, in this study we will use CRC cells to analyze post-translational modification and known gene target of STAT3 through Western Blot and RT-PCR, after treatments with Nexturastat A (NextA), a specific HDAC6 inhibitor, that indirectly decreases the activation levels of STAT3, this will allow observe the STAT3 modifications and quantify the expression of IL-10 and PD-L1. Then, to simulate a pro-inflammatory environment, we treated cells with interleukin-6 (IL-6) and NextA, analyzed by Western blot and flow cytometry. Finally, we analyzed RNAseq after IL-6/NextA treatment, that showed a specific expression of genes involved in immunomodulatory pathways. These results allow us to suggest that treatment with specific HDAC6 inhibitors triggers changes in STAT3 phosphorylation in CRC cells and generates a change in the expression of immunomodulatory genes involved in the immune response. In conclusion, HDAC6 inhibition may be suggest as a new therapeutic target for Colorectal Cancer.

Funding: This work was supported by National Research and Development Agency, through FONDECYT iniciacion (11190287) and UCSC (FAA 01/2022) to MIH. Direccion de Postgrado UCSC, through Beca Apoyo a Actividades Académicas Primera Convocatoria año 2022 to CM.

"Histatin-1, a pro-angiogenic factor that promotes endothelial cell migration via VEGFR2"

Carlos Mateluna[1], Pedro Torres[1], Patricio Silva[1], Marcelo Rodríguez[1], Douglas Matthies[2], Floris Bikker[3], Christian Wilson[4], Gerald Zapata[2], **Vicente A. Torres[1,5,6]**

[1]Institute for Research in Dental Sciences, Faculty of Dentistry, Universidad de Chile, Santiago, Chile; [2]Molecular Graphics Suite, Department of Inorganic and Analytical Chemistry, Faculty of Chemical and Pharmaceutical Sciences, Universidad de Chile, Santiago, Chile; [3]Department of Oral Biochemistry, Academic Centre for Dentistry Amsterdam, VU University & University of Amsterdam, Netherlands; [4]Department of Biochemistry and Molecular Biology, Faculty of Chemical and Pharmaceutical Sciences, Universidad de Chile, Santiago, Chile; [5]Advanced Center for Chronic Diseases (ACCDiS); [6]Millennium Institute on Immunology and Immunotherapy (MIII).

Histatins are salivary peptides that play important roles in the maintenance of enamel and oral mucosal homeostasis. From this group of peptides, Histatin-1 stands out, as it has been shown to promote wound healing in different epithelial tissues, by stimulating the movement of keratinocytes, fibroblasts and endothelial cells. Particularly, in the latter model, our group demonstrated that Histatin-1 is a potent pro-angiogenic factor that increases both endothelial cell adhesion and migration, leading to vascular morphogenesis in vitro and angiogenesis in vivo. However, despite the knowledge about the mode of action of Histatin-1, the mechanism of action by which this peptide induces angiogenesis remains largely unknown. In this context, we recently identified the receptor for Histatin-1 in endothelial cells, VEGFR2 (Vascular Endothelial Growth Factor Receptor 2), which spatially co-distributed, associated in a protein complex and interacted with Histatin-1 in vitro. By using pulldown assays with pure, tagged and non-tagged proteins, Histatin-1 and VEGFR2 were shown to directly interact, and this interaction was transient, specific and direct, as determined by optical tweezers and molecular dynamics. Moreover, we found that residues Phe26, Tyr30 and Tyr34 of Histatin-1 were critical for VEGFR2 binding and activation, as shown by molecular dynamics and in vitro binding assays. Accordingly, alanine mutation of these residues prevented Histatin-1-induced endothelial cell migration and angiogenesis. Collectively, our studies show that VEGFR2 is the Histatin-1 receptor in endothelial cells and provide insights to the mechanism by which this peptide promotes endothelial cell migration and angiogenesis.

Funding: This work was supported by FONDECYT 1220517 (VT), 1171484 (GZ), 1181361 (CW); FONDAP 15130011 (VT); Iniciativa Científica Milenio ICN09_016/ICN 2021 (VT); ANID fellowship 21181094 (CM).

"The primary cilium is required for palmitic acid-induced inflammation in hypothalamic neurons"

Catalina Kretschmar[1,2,3], María Paz Hernández-Cáceres[1,3], Daniel Peña-Oyarzún[2,3,4,5], Eugenia Morselli[3,6], Alfredo Criollo[1,2,3]

[1] Instituto de Investigación en Ciencias Odontológicas, Facultad de Odontología, Universidad de Chile
[2] Advanced Center for Chronic Diseases (ACCDiS), University of Chile, Santiago, Chile.
[3] Autophagy Research Center, Universidad de Chile
[4] Physiology Department, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, Chile.
[5] 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
[6] Department of Basic Sciences, Faculty of Medicine and Sciences, Universidad San Sebastián, Santiago de Chile

Long-term high-fat diet consumption leads to hypothalamic inflammation and deregulation of energy balance. Primary cilium (cilia), a single non-motile organelle that protrudes from the surface of most cell types is described as a key organelle for the integration of signals that control energy homeostasis. Thus, cilia dysfunction in hypothalamic neurons is associated to alterations in metabolic homeostasis. We have recently established that palmitic acid (PA), the main saturated fatty acid consumed in modern diets, induces cilia loss and shortening in hypothalamic neurons. In this regard, recent studies have proposed that releasing of extracellular vesicles (EVs) from cilia could be a mechanism that drives cilia shortening. Thus, we evaluated if PA induces ciliary EVs release from hypothalamic neurons. Immunofluorescence analyses shows that the inhibition of EVs release from cilia by using cytochalasin D blunts PA-induced cilia loss and shortening. To evaluate the effect of PA specifically in cilia, we generated the hypothalamic cilia-KO stable cell line, GT1-7-CEP164-/-. EVs isolation from media of GT1-7 WT and GT1-7-CEP164-/- showed an increase in EV release in response to PA only in GT1-7 WT, suggesting that EVs derive from cilia. Then, to investigate the pathological effects of PA-induced cilia loss, we assessed the inflammatory response through the activation of NF-kB singling pathway. Our data revealed an increase in the levels of p-IkB-α as well as upregulation in IL-6 gene expression in response to PA, which were abolished in GT1-7-CEP164-/- cells. Altogether, our studies suggest that cilia are required to promote PA-induced inflammation in hypothalamic neurons.

Funding: This project was funded by FONDECYT 1211329 (AC) and 1200499 (EM), and ANID doctoral Fellowship 21191773 (CK).

"Chilean colorectal cancer is characterized by a dysbiosis and the presence of *Fusobacterium nucleatum*"

Roxana Gonzalez-Stegmaier[1], Troy Ejsmentewicz[1], Roddy Jorquera[1], Rodrigo Lagos[2] María Cristina García[3], Celia Podesta[3], Carlos Barrientos[3], Franz Villarroel-Espíndola[1]

[1]Gastrointestinal Immuno-oncology group, Translational Medicine Research Laboratory. Instituto Oncológico Fundación Arturo López Pérez (FALP), Santiago, Chile.
[2]Clinical Studies and Epidemiology Unit. Instituto Oncológico Fundación Arturo López Pérez (FALP), Santiago, Chile.
[3]Endoscopy Unit. Instituto Oncológico Fundación Arturo López Pérez (FALP), Santiago, Chile.

As the third most common malignancy and the second most deadly cancer, colorectal cancer (CRC) induces estimated 1.9 million incidence cases and 0.9 million deaths worldwide in 2020. The role of gut microbiome in CRC is influenced by the composition of microbiota and the abundance of specific species. The dysbiosis or alteration on microbiome balance is associated with an increased risk of CRC. To understand the role of gut microbiome in Chilean CRC, an ongoing prospective clinical study from a single center was designed in FALP. Interim analysis included 28 participants enrolled during 2020 and 2021. Microbiome composition from stool was determined by metagenomic 16S gene sequencing and bioinformatic analysis. For the CRC group (N=8), the results showed a significantly reduced relative abundance of *Firmicutes* ($p<0.05$) compared with the control group, and a significant presence of *Fusobacteria* phylum compared with a control group without cancer (N=9), or a group with polyps (N=10). In addition, 7 out of 8 CRC cases had *Fusobacterium nucleatum or Fusobacterium spp.*, which was confirmed by compositional data analysis (adjusted p-value≤0.1). No significant differences were observed at phylum level, between patients with an active cancer or previously operated; however, when alpha diversity was estimated, it was significantly lower in operated patients ($p<0.05$). Dysbiosis and *Fusobacterium* within the gut microbiome are significantly associated with CRC, and both events are observed within the local population. More participants and investigations are required to understand the role of gut microbiome as a promotor of intestinal carcinogenesis.

Funding: This work was supported by The National Fund for Scientific and Technological Development, FONDECYT (N° 1221415 to FVE) and Internal grant from Fundación Arturo López Pérez (FALP-LMT-2020, -2021 to FVE, and -2022 to RGS).

"Effect of Nordihydroguaiaretic acid (NDGA) on lung cancer cell viability"

Mónica Salas[1], Carina Chipón[1,2], Rodrigo López[2], Angara Zambrano[1]

[1] Laboratorio de Epigenética, Biología Celular y Productos Naturales, Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile.
[2] Laboratorio de Farmacología y Fisiopatología Pulmonar, Instituto de Farmacología y Morfofisiología, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Valdivia, Chile.

Lung cancer is the leading cause of death from cancer in Chile and the world. Despite efforts to improve current therapies, there is still a need to look for new therapeutic options that can enhance the effectiveness of treatments, context where natural products have been a massive focus of attention in recent years. The primary purpose of this work was to demonstrate the effects of nordihydroguaiaretic acid (NDGA), the main metabolite of the Cresote bush, on lung cancer cell lines. For this, were carried out resazurin reduction viability assays in seven lung cancer cell lines, where it was observed that NDGA produces a drastic decrease in the survival of these cells after 24, 48 and 72h of treatment. Likewise, the impact of this natural drug has also been corroborated through others viability assays as the use of a nucleic acid binding probe (propidium iodide) and cell apoptosis studies. The results indicate that this polyphenol could significantly impact lung cancer treatment. Further studies will enable us to identify the mechanism of action of this lignan and if it can be used as an adjuvant in current therapies against lung cancer.

Funding: This work was support by Fondecyt Regular 1141067 and 1180957, and VIDCA from Universidad Austral de Chile.

The autophagy process as a modulator of senescence associated secretory phenotype in senescent gastric cancer cells.

Claudio Valenzuela[1], Angel Cayo[1,2], Whitney Venturini[1,2], Raúl Segovia[1,2], Danitza Rebolledo[1], Rodrigo Moore-Carrasco[2], Nelson Brown[1]

[1] Center for Medical Research, University of Talca Medical School, Talca, Chile.
[2] Faculty of Health Sciences, University of Talca, Talca, Chile.

Cellular senescence corresponds to a permanent cell cycle exit triggered by several stimuli, including therapeutic stress associated with anti-neoplastic drugs that block the cell cycle progression, such as inhibitors of cyclin 4 or 6-dependent kinases. (CDK4/6). In addition to the cessation of proliferative activity, the establishment of the senescent phenotype is associated with extensive metabolic reprogramming and the acquisition of the ability to secrete bioactive molecules into the extracellular environment (SASP). Paradoxically, the SASP can promote the generation of an inflammatory microenvironment that, maintained chronically, may facilitate the neoplastic transformation of neighbor cells. Another characteristic of senescent cells is autophagy induction, a catabolic process through which lysosomal enzymes degrade cellular components. Although autophagy acts as a quality control mechanism in basal conditions or as a survival mechanism in conditions of nutrient deprivation, the role of autophagy in cellular senescence has been challenging to establish. In order to determine the autophagy role in the senescent secretory phenotype modulation, the senescence process, with or without the concomitant autophagy inhibition, was pharmacologically triggered by inhibition of CDK4/6 activity with 0,5-1μM of Palbociclib (PD0332991) for 48 and 96 hours. Autophagy was known down by the addition of short hairpin (shATG5) or inhibited by 0,5μM Spautin-1. The autophagy inhibition decreases the expression of some molecules induced by senescent. Moreover, it exacerbates decreased glucose uptake into the cell and lactate production. This indicates that autophagy is a mechanism necessary for SASP implementation. The next will be to determine the effect on the tumor microenvironment.

Funding: This work was supported by Fondecyt (11201182)

"SALL2 expression in colon cancer progression and its association with the WNT/β-catenin pathway".

Aracelly Quiroz[1] , Constanza Mardones[1], José Miguel Navarrete[1], Ángela Ortíz[1] Paula Medina[1], Víctor Fica[2] ,Alexis Salas[2], Carolina Delgado[3], Ariel Castro[1], Roxana Pincheira[1]

[1]Laboratorio de Transducción de señales y Cáncer, departamento de Bioquímica y Biología Molecular, Universidad de Concepción.
[2]Laboratorio de Diseño Racional de Fármacos, departamento de Farmacología, Universidad de Concepción.
[3]Unidad de Anatomía Patológica, Hospital Guillermo Grant Benavente.

SALL2 is a developmental transcription factor involved in the regulation of cell proliferation, migration and survival. Massive analyses indicated that *SALL2* mRNA significantly decreases in colorectal cancer (CRC), a cancer type characterized by hyperactivation of the WNT pathway. Interestingly, our unpublished ChIP-seq data analyses suggested that SALL2 regulates genes associated with the WNT pathway. However, the role of SALL2 in CRC and its relationship with the WNT pathway are yet unknown. We used tissue microarray of 130 samples from Guillermo Gantt Benavente's Hospital comprising normal colon, adenoma, and CRC to evaluate the expression and cellular location of SALL2 using chromogenic immunohistochemistry and multiplexed immunofluorescence. We found that SALL2 expresses in the nucleus and cytoplasm of the normal colon epithelium and the stroma. However, its expression decreases in adenoma and is absent in CRC. We noted a negative correlation between SALL2 expression and the expression of nuclear β-catenin, at the migratory front. Subcellular fractionation and immunofluorescence experiments in CRC cells with gain and loss of SALL2 function also support the negative correlation between SALL2 expression and nuclear β-catenin. Additionally, we evaluated the expression of WNT targets by Western blot and qRT-PCR, finding a positive correlation between SALL2 and AXIN2, a negative regulator of the WNT pathway. Analysis of AXIN2 promoter identified several putative SALL2 binding sites, and SALL2 increases *AXIN2* promoter activity, suggesting a SALL2- dependent transcriptional regulation. Besides, we confirm that SALL2 binds to *AXIN2* promoter, using ChIP-qPCR.

Finally, we show that the SALL2-AXIN2 axis is required for the survival response to XAV939, an inhibitor of the Wnt pathway. Our study shows that SALL2 positively regulates the transcription of AXIN2. We proposed that the loss of SALL2 expression during CRC progression contributes to the decrease of AXIN2 levels, the hyperactivation of the WTN pathway, and the malignant phenotype.

Funding: FONDECYT grants 1191172 and 1201215

"LigRMSD: A web server for analyzing the quality of protein-ligand molecular docking results"

Julio Caballero[1], José Luis Velázquez–Libera [1], Fabio Durán-Verdugo [1], Alejandro Valdés-Jiménez [1], Gabriel Núñez-Vivanco [1]

[1]Center for Bioinformatics, Simulations and Modelling, Universidad de Talca, Talca 3460000, Chile

Many proteins in biochemical pathways associated with specific diseases can be modulated by small molecules or ligands, potentially causing relief or complete cure to patients. For this reason, the study of protein-ligand interactions is carried out with great effort to find novel drugs. Molecular docking has been consolidated as one of the essential methods in the molecular modeling field to characterize protein-ligand interactions. Although docking methods allow for finding adequate solutions, they also provide multiple wrong poses, so the comparison with a reference is recommended when proposing a result. Root mean square deviation (RMSD) is one of the most valuable and straightforward measures for structural comparison between calculated conformations and a reference. Commonly, protein-ligand docking programs have included some utilities that allow calculating this value; however, they only work efficiently for identical (not similar) compounds when a complete atom label equivalence exists between the evaluated conformations. We present LigRMSD, a free web-server for the automatic matching and RMSD calculations among identical or similar chemical compounds. This server allows users to submit a pair of identical or similar molecules or a dataset of similar compounds (without necessarily having a complete atom label equivalence) to compare their three-dimensional conformations. LigRMSD can be freely accessed at https://ligrmsd.appsbio.utalca.cl (this tool was published in Bioinformatics 2020, 36, 2912-2914, DOI:10.1093/bioinformatics/btaa018).

Funding: The assistance to the Annual Meeting of the Society for Biochemistry and Molecular Biology of Chile 2022 was supported by the Fondo Nacional de Desarrollo Cientifico y Tecnologico (FONDECYT) [1210138]. The web server is hosted in the cluster obtained with the grant [CONICYT-FONDEQUIP-EQM160063].

"Design of inhibitory peptides based on molecular modeling of the interaction TRPM4-KCTD5"

Javiera Baeza[1,3], Wendy González[1,3], Oscar Cerda[2,3]

[1]Centro de Bioinformática, Simulación y Modelado, Facultad de Ingeniería, Universidad de Talca, Talca, Chile.
[2]Programa de Biología Celular y Molecular, Instituto de Ciencias Biomédicas (ICBM), Facultad de Medicina, Universidad de Chile, Santiago, Chile.
[3]Millennium Nucleus of Ion Channels-Associated Diseases (MiNICAD).

The transient potential melastatin (TRPM) family members have emerged as a group of ion channels with high biomedical potential due to their involvement in several physiological functions. Mutations in the genes encoding TRPM channels are linked to different channelopathies. High TRPM4 expression has been reported to be associated with pathologies that alter cytoskeletal rearrangement and cell migration, such as fibrosis and metastatic cancer (G4 stage) in different cell lines. KCTD5 was identified as a positive regulator of TRPM4 that causes an increased Ca+2 sensitivity. It is involved in cell migration through TRPM4 regulation. TRPM4 and KCTD5 expression levels are elevated in breast cancers with poor prognosis. Therefore, TRPM4-KCTD5 interaction represents an attractive target for the development of drugs that modulate TRPM4 activity with a therapeutic potential. We propose to design and evaluate interfering peptides based on TRPM4-KCTD5 interaction using molecular modeling and molecular dynamics techniques, Free energy calculations (MMGBSA) and in vitro assays to elucidate their involvement in advanced clinical stage of breast cancer.

Funding: This work was supported by Fondo Nacional de Desarrollo Científico y Tecnológico (Fondecyt N° 1191133).

"Comprehensive re-analysis of hairpin RNAs in fungi shows ancestral links"

Nathan R. Johnson[1,2], Luis F. Larrondo[1,4], José M. Álvarez[1,2,5*], Elena A. Vidal[1,2,3*]

[1]Millennium Science Initiative – Millennium Institute for Integrative Biology (iBio), Santiago, Chile
[2] Centro de Genómica y Bioinformática, Facultad de Ciencias, Ingeniería y Tecnología, Universidad Mayor, Santiago, Chile
[3]Escuela de Biotecnología, Facultad de Ciencias, Universidad Mayor, Santiago, Chile
[4]Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile
5Centro de Biotecnología Vegetal, Facultad de Ciencias, Universidad Andrés Bello, Santiago, Chile

RNA interference is an ancient mechanism with many regulatory roles in eukaryotic genomes, with small RNAs acting as their functional element. While there is a wide array of classes of small-RNA-producing loci, those resulting from stem-loop structures (hairpins) have had a particular focus. Such is the case of microRNAs (miRNAs), which have distinct roles in plants and animals. Fungi are also able to produce small RNAs, and several publications have identified miRNA-like hairpin RNAs in diverse fungal species, using deep sequencing technologies. Despite this relevant source of information, to date relatively little is known about miRNA-like features in fungi, mostly due to a lack of established criteria for their annotation. To systematically assess miRNA-like characteristics and quality, we re-assessed the annotations from available publications with small-RNA-seq data from 40 fungal species. We extracted and normalized the annotation data for 1,682 reported miRNA-like loci and determined their small RNA abundance profiles, showing that less than half of the reported loci reached stranding and abundance standards. We found that fungal hairpins are generally more similar in size to animal miRNAs and frequently were associated with protein-coding regions of the genome. Genomic searches identified 18 hairpin loci likely conserved in multiple species. Our pipeline allowed us to build a general hierarchy of locus quality, identifying around 200 loci with high-quality annotations. We provide a centralized annotation of identified hairpin RNAs in fungi and is an important step in understanding the characteristics and functions of miRNA-like small RNA classes in fungal organisms.

Funding: This work was supported by the National Agency for Research and Development of Chile (ANID) FONDECYT program (number 11220727); and the ANID - Millennium Science Initiative Program (ICN17_022).

"A workflow to compare ligand binding sites from molecular dynamics simulations"

José Carlos Estanislao Márquez Montesinos[1], Yuliet Mazola Reyes[1], Wendy González Díaz [1,2]

[1] Center for Bioinformatics, Simulation and Modeling (CBSM), Universidad de Talca, Chile.
[2] Millennium Nucleus of Ion Channels-Associated Diseases (MiNICAD), Chile.

Introduction: Polypharmacology is a modern drug research paradigm that can be used to tackle complex diseases, taking advantage that several molecules are involved in the pathology. This criterion focuses on the design and development of a drug that might interact and modulate several key targets involved in the same pathology, *i.e.*, multi-target directed ligands (MTDL). The binding site (BS) comparison is meaningful to assist the rational MTDL design and there are several tools to address this task. However, to the best of our knowledge, there is no protocol that can take advantage of molecular dynamics (MD) data to fulfill the comparison of BSs. Hence, A computational workflow is presented to achieve the BS comparison using information from MD. Methods: The workflow has to main sections: (1) BS characterization and (2) BS comparison. The step (1) includes Fpocket physicochemical features and contact residues frequency calculation, and a prediction of PLIP interaction profile. Next, the physicochemical data of the BS is used to cluster and retrieve the representative frames from the MD. The step (2) takes the BS of the representative frames and compare them by pairs using PocketMatch, selecting the best match between the systems. Finally, a BS structural alignment is performed by PocketAlign, using the information of contact residues frequency and the interaction profile calculations. Discussion and conclusion: MDs from Kv1.5 and Nav1.5 channels bound with the antiarrhythmic flecainide were used to prove the concept. A common structural pattern for flecainide BS was found, revealing two matching areas: a hydrophobic patch and a polar region. Moreover, a distinctive feature was identified in Nav1.5, which could be responsible for the higher affinity presented by flecainide. This workflow is intended to be used in rational MTDL design and could be extended for a comparison of more than two systems.

Keywords: Multi-target; drug promiscuity; druggable binding site; flecainide; Nav1.5; Kv1.5; binding site comparison; polypharmacology

Funding: This research was funded by Fondo Nacional de Desarrollo Científico, Tecnológico y de Innovación Tecnológica (FONDECYT) grant number 1191133 and Millennium Nucleus of Ion Channel Associated Diseases (Minicad).

"Rational design of peptide-based functional biomaterials to enhance molecular recognition"

Horacio Poblete[1,2] , Ariela Vergara-Jaque[1,2] , Cristian Vilos[2]

[1] Center for Bioinformatics, Simulation and Modeling. Universidad de Talca, Chile.
[2] Center for Nanomedicine, Diagnostic & Drug Development, Universidad de Talca, Chile.

Cancer is one of the biomedical conditions that courses with unlike pathophysiological development; however, the use of targeting strategies focused on integrins could be a unique one-way opportunity to find innovative therapeutics endeavors. Our primary goal is to determine, through a high-throughput computational framework, how rationally designed peptides can enhance the recognition and release of antitumoral drugs loaded on polymeric nanoparticles for colorectal cancer. Through a computational approach, it has been possible to model the association of nanoparticles with peptide-based structures and to evaluate their affinity by protein receptors, as well as the transmembrane diffusion of cancer drugs (CD) needed for cancer-cell elimination. Here we have been putting together the puzzle identifying: (1) The force profile of the permeation of Irinotecan, Cisplatin, and the dye Rhodamine B from polymeric nanoparticles. (2) We have identified a small sequence of peptides highly selective by Integrins receptors that are over-expressed in colorectal cancer. (3) An estimation of the free-energy profile of the permeability of cancer drugs on different membrane models.

Funding: This work was supported by the Fondecyt Regular Nº, 1211143, 1220110, and 1201147

"Analysis of the allosteric regulation by AMP in enzymes from methanogenic archaea"

Felipe Gonzalez-Ordenes[1], Nicolas Herrera[1], Víctor Castro-Fernandez[1], Victoria Guixé[1]

[1]Universidad de Chile, Departamento de Biología, Facultad de Ciencias, Las Palmeras 3425, Ñuñoa, Chile.

Although it has been claimed that enzyme regulation in archaea occurs mainly at the transcriptional level, some examples of AMP allosteric regulation in archaea have been recently reported. To identify novel regulatory points in central carbon metabolism, we kinetically and structurally characterized three enzymes from the model organism *M. maripaludis*: glycogen phosphorylase (Mm-GP), fructose 1,6 bisphosphatase aldolase (Mm-FBPA), and phosphoenolpyruvate synthase (Mm-PEPS). Canonical glycogen phosphorylases catalyze the first step in the breakdown of glycogen using pyridoxal phosphate (PLP). This cofactor is covalently bound by a strictly conserved active site lysine. Multiple sequence alignment revealed that in Mm-GP, this lysine is replaced by a threonine. Analysis of PLP content by fluorescence and mass spectrometry in Mm-GP shows the absence of this cofactor, although the enzyme is catalytically active, prefers branched glucose polymers and is not regulated by AMP. Mm-FBPA is a bifunctional enzyme that is only active in the presence of a high concentration (50 mM) of reducing agents and is regulated by AMP. While Mm-PEPS revealed the most striking structural features, as indicated by the size exclusion analysis that revealed the formation of a homo-oligomer complex of 1.2 MDa of unknown structure. Our results indicate that the central carbon metabolism of *M. maripaludis* is regulated by AMP at the F1,6BP to fructose-6P step and reveals novel structural features exclusively present in archaeal enzymes.

Funding: Proyecto Fondecyt regular 1191321 y Fondecyt 1221667

"Structural, functional characterization and engineering of PET hydrolases from polar marine environments"

Aransa Griñen[1,2], Jerónimo Cifuentes-Anticevic[3], Paula Blázquez-Sánchez[1,2], Jhon A. Vargas[4], Adriano A. Furtado[4], Felipe Engelberger[1,2], Felipe Gatica[1,2], Humberto M. Pereira[4], Richard C. Garratt[4], Beatriz Díez[3,5,6], César A. Ramírez-Sarmiento[1,2]

[1] Institute for Biological and Medical Engineering, Schools of Engineering, Medicine and Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, Chile
[2] ANID – Millennium Science Initiative Program – Millennium Institute for Integrative Biology (iBio), Santiago, Chile
[3] Department of Molecular Genetics and Microbiology, School of Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, Chile
[4] Instituto de Física de São Carlos, Universidade de São Paulo, São Carlos, Brazil
[5] Center for Climate and Resilience Research (CR)2, Santiago, Chile
[6] FONDAP Center for Genome Regulation (CGR), Santiago, Chile

Polyethylene terephthalate (PET) represents 10% of the annual production of plastics worldwide, accumulating as waste at rates paralleling its production level and making up to 90% of marine waste. However, the discovery of PET hydrolases (PETases) that efficiently degrade this plastic has paved the way for the potential biological recycling of PET. Despite recent advances in their identification and characterization from terrestrial and marine environments, the search for natural PETases at moderate temperatures is a challenging task. In this regard, we recently employed bioinformatic approaches to identify many potential PETases from Antarctic bacteria and polar marine metagenomes. Building up from this prior work, here we present the functional and structural characterization of such enzymes. First, we present the functional characterization of Mors1 from the Antarctic bacterium Moraxella sp. TA144 against polycaprolactone (PCL) and PET at 25 ºC and its recently solved experimental structure, which provides evidence of the increase in disulfide bonds in Antarctic PETases to maintain activity at low temperatures. Second, we present the structural and functional characterization of two metagenomic enzymes, metaG4 and metaG7, with the latter exhibiting hydrolytic activity against PCL. Lastly, we present the engineering of Mors1 to optimize its PET-degrading activity by swapping of active site loops from thermophilic PETases, leading to a 4-fold increase in PET hydrolysis and 20 ºC in optimal temperature for such activity. Altogether, these results provide insights for understanding how these enzymes degrade PET at low temperatures.

Funding: ANID-FAPESP 2019/13259-9, ANID Millennium Science Initiative Program ICN17_022, ANID PFCHA 21220450

"Bioinformatic characterization of exoglycosidase lysosomal alpha-mannosidase involved in fruit ripening of *Fragaria x ananassa*"

Angela Méndez-Yáñez [1], Claudio Letelier-Naritelli [2], Yessica Rojas-Burgos [3], Felipe Valenzuela-Riffo [4] and Luis Morales-Quintana[1]

[1] Multidisciplinary Agroindustry Research Laboratory, Instituto de Ciencias Biomédicas, Facultad de Ciencias de la Salud, Universidad Autónoma de Chile, Talca, Chile.
[2] Escuela de Ingeniería Civil en Bioinformática, Facultad de Ingeniería, Universidad de Talca, Chile.
[3] Escuela de Ingeniería en Biotecnología, Facultad de Ciencias Agrarias y Forestales, Universidad Católica del Maule, Talca, Chile.
[4] Laboratorio de Fisiología Molecular Vegetal, Instituto de Ciencias Biológicas, Universidad de Talca, Chile.

Lysosomal alpha-mannosidases (α-Man; EC 3.2.1.24) are relevant family enzyme in human health, involved in alpha-mannosidosis disease, a rare genetic condition. This exoglycosidase in plants is involved in the fruit ripening process hydrolyzing α-1,2, α-1,3 and α-1,6 glycosidic linkages in non-reducing alpha-D-mannose residues in N-glycosylations, promoting indirectly the softening of fleshy fruit. Experimental evidence in RNA interference of α-Man in tomato and capsicum maintain fruit without deterioration until 45 days and 15 days, respectively.
Fragaria x ananassa, is an important non climacteric fruit commercialized in Chile and the world, and used as study model in development and ripening process. Additionally, cell wall enzymes, with activity on polysaccharides, has been studied and related with softening and loosening process. Many of these enzymes are N-glycosylated, being N-deglycosylated by enzymes as α-Man. As consequence of this hydrolysis, free N-glycans of this reaction could be act a signal molecules promoting the ripening.
According with the last, we carry out a bioinformatic characterization of this family in *F. x ananassa* was elaborated to understand their gene structure, phylogenetic classification, and conserved domain. Also, their enzymatic activity was quantified in fruit developmental stage. New insights are suggested to applications in vegetal biotechnology.

Funding: This work was supported by Agencia Nacional de Investigación y Desarrollo de Chile [FONDECYT Postdoctoral #3220284 to A.M-Y.; Regular FONDECYT #1220782 to L.M-Q].

"Implementing an optogenetic intercellular communication system in yeast"

Vicente Rojas[1,2], Luis F. Larrondo[1,2]

[1] Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Chile.
[2] Instituto Milenio de Biología Integrativa (iBio), Chile.

Cell communication is a widespread mechanism in biology, allowing the transmission of information about environmental conditions. In order to understand how cell communication modulates relevant biological processes (such as survival, division, differentiation or apoptosis), different synthetic systems based on chemical induction have been successfully developed in prokaryotic and eukaryotic microbial platforms. In this work, we coupled cell communication and optogenetics in the budding yeast Saccharomyces cerevisiae. Our approach is based on two strains connected by the light-dependent production of α-factor pheromone in one cell type, which induces gene expression in the other type. After the individual characterization of the different variants of both strains, the optogenetic intercellular communication system was evaluated by combining the cells under contrasting illumination conditions. Using luciferase as a reporter gene, specific co-cultures at 1:1 ratio displayed activation of the response upon constant blue-light, which was not observed for the same cell mixtures grown in darkness. Then, the system was assessed at several dark/blue-light transitions, where the response level varies depending on the moment in which illumination was delivered. Furthermore, we observed that the amplitude of the response can be tuned by modifying the initial ratio between both strains. Finally, the two-strain system showed higher fold-inductions in comparison with autonomous strains. Altogether, these results demonstrated that external light information is propagated through a diffusible signaling molecule to modulate gene expression in a synthetic system involving microbial cells, which will pave the road for studies allowing optogenetic control of population-level dynamics.

This work was supported by ANID-Ph.D. scholarship 21170331, ANID-FONDECYT 1211715, iBio and HHMI International Research Scholar grant.

"Histone H3 demethylase KDM5A regulates key genes in early lineage commitment from human pluripotent stem cells to neural progenitors"

Estefania Cardona[1], Christopher Fierro[1], Alejandro Blanco[1], Victor Pola[1], Brigitte van Zundert[1], Martin Montecino[1]

[1]Institute of Biomedical Sciences, Faculty of Medicine and Faculty of Life Sciences, Universidad Andrés Bello, Santiago, Chile

Commitment of human induced pluripotency cells (hiPSCs) to neural progenitors (hNPCs), involves transcriptional changes regulated through epigenetic mechanisms, including the histone post-translational modification tri-methylation of lysine residue 4 in histone 3 (H3K4me3). This mark is associated with transcription activity [mostly enriched at the transcription start sites (TSS)] and has been shown to play a critical role in cell differentiation. Demethylase 5A (KDM5A) removes this mark and hence contributes to reduce gene expression. The role of KDM5A in the early commitment to hNPCs has not been determined. Here, we identified genes regulated by KDM5A during the differentiation of hiPSCs to hNPCs and examined, by RNA sequencing, whether their transcriptional activity correlates with the levels of H3K4me3 at the TSS. The distribution of KDM5A and H3K4me3 throughout the genome in hiPSCs and hNPCs was determined by chromatin immunoprecipitation coupled with DNA sequencing (ChIPseq). We find that KDM5A enrichment at the TSS changes during differentiation and that is associated with transcriptional activity. A set of genes show KDM5A gain, decreased expression, together with reduced H3K4me3 enrichment. However, other genes exhibit increased KDM5A binding and enhanced expression. Also, we identified genes that lose KDM5A and simultaneously gain transcriptional activity and H3K4me3 enrichment. Interestingly, among them, is the KDM5A gene itself. Together our results reveal that KDM5A may function both in the canonical and in a non-canonical fashion during differentiation to hNPC. Importantly, binding of KDM5A to its own gene promoter suggests a self-regulatory mechanism to control KDM5A expression during this early differentiation process.

Funding: FONDECYT1211026

"Epigenetics effects of obesity in the neurons that regulate feeding behavior"

Estefanía Tarifeño-Saldivia[1] , Valentina Opazo[1], José Medina[1] , Isidora Manriquez[1] Daniel Candia[1], Patricio Ordenes[2], Paula Llanos[3], María de los Ángeles García[2]

[1] Gene Expression and Regulation laboratory (GEaRLab), Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción
[2] Laboratorio de Biología Celular, Departamento de Biología Celular, Facultad de Ciencias Biológicas, Universidad de Concepción.
[3] Bioengineering Laboratory, Universidad Adolfo Ibáñez.

Obesity is a chronic medical condition characterized by increased body weight and excess body fat. In Chile, 74% of the adult population suffers from overweight, and 34,4% of obesity. This metabolic condition produces systemic effects among which the neuronal circuit regulating feeding behavior is dramatically disrupted. Indeed, it has been postulated that macronutrients present in obesogenic diets (such as saturated fatty acids) induce specific effects in the hypothalamic POMC neurons such as leptin resistance, dramatic transcriptomic modulation, and loss of these neurons, leading to failure to control hyperphagia in obese mice. Long-lasting effects might be supported by epigenetic modifications - induced by obesity - that maintain gene dysregulation even after normal weight has been achieved. It has been demonstrated that obesity induces DNA hypermethylation on the Pomc promoter of POMC neurons disrupting the normal leptin signaling, but genome-wide effects have not been yet evaluated which might explain the dramatic transcriptome modulation documented for these neurons. In this work, we studied the effects of a long-term high-fat diet on the methylome of FACS-purified POMC neurons to identify gene modulation induced by methylation dynamics. These results will open new research lines regarding the functional role of these genes in rebound weight gain and the discovery of alternative therapies. Such kind of research is of fundamental interest to our country, as Chile is becoming an obese population.

Funding: This work has been funded by Fondecyt Iniciación N°11190401 (ANID/FONDECYT), VRID-Iniciación 218.037.024-INI (Universidad de Concepción)

"BCL6: A novel transcription factor involved in cardiac hypertrophy revealed trough human transcriptional regulatory networks"

Juan Francisco Silva-Agüero[1], Victor Aliaga-Tobar[2], Ximena Calle[1], Jafet Ortiz-Quintero[1,3] Mauricio Latorre[2,4,5], Valentina Parra[1,5,6]

[1] Department of Biochemistry and Molecular Biology and Advanced Center for Chronic Diseases (ACCDiS), Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile.
[2] Bioengineering Laboratory; Institute of Engineering Sciences; O'Higgins University, Rancagua, Chile.
[3] Department of Bioanalysis and immunology, Faculty of Science, National Autonomous University of Honduras. Tegucigalpa, Honduras
[4] Laboratory of Bioinformatics and Gene Expression, INTA, University of Chile, Santiago, Chile.
[5] Systems biology center for the study of extremophile communities from mining tailings (SYSTEMIX), O'Higgins University, Rancagua, Chile.
[6] Network for the Study of High-lethality Cardiopulmonary Diseases (REECPAL), University of Chile, Santiago, Chile.

Heart failure (HF) is a complex condition where the heart cannot pump enough blood to the body. HF onset is preceded by cardiac hypertrophy (CH), an adaptative response to increased workload characterized by cardiomyocyte enlargement. Cardiac stressors such as Norepinephrine (NE) activate a global transcriptional response, involving different signaling cascades of master transcription factors (TFs, e.g., NFAT, GATA4, MEF2). Using a systems biology approach, we build a human genome-scale transcriptional regulatory network (TRN) to identify novel TF involved in CH. To this end, we first selected different public articles and databases to consolidate a human TRN template that comprises TF-TF and TF-gene interactions. Second, we analyze public human HF transcriptomic datasets (RNAseq) to obtain differentially expressed genes. By integrating HF global gene expression in the human TRN, we generated a model of TRN activated by HF. Interestingly, the network contains several TF involved in CH/HF in addition to novel uncharacterized factors, selecting BCL6 as a putative new regulator. To evaluate BCL6 response under CH conditions, we treated neonatal rat cardiomyocytes (NRVM) with NE, observing an increase in BCL6 mRNA and protein levels, correlated with the overexpression of classical hypertrophy marker genes (ANP, BNP, and β-MHC). Moreover, BCL6 knockdown ameliorated the overexpression of ANP and BNP of NRVM treated with NE. This is the first report showing a human TRN for HF, a model capable of identifying interesting new TF involved in CH. Finally, our results suggest that BCL6 may exert a pro-hypertrophic role on the NE-induced CH model.

Funding: This project was funded by FONDECYT 1190743 (VP) and 1190742 (ML), ICGEB CRP/CHL18-04 (VP, ML), U-Redes G_2018-35 (VP), ANID FONDAP 15130011 (VP), ANID BASAL FB210005 (ML), ANID MILENIO ICN2021_044 (ML), ANID ANILLO ACT210004 (ML) and ACT20210044 (VP, ML).

"The underlying regulatory circuit behind the diauxic lag-phase duration in *Saccharomyces eubayanus*"

Felipe Muñoz-Guzmán[1,2], Pablo Quintrel[1,2], Tomas A. Peña[1,2], Francisco A. Cubillos[1,2]

Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile.
ANID-Millennium Science Initiative-Millennium Institute for Integrative Biology (iBio), Santiago, Chile.

Yeast growing under two carbon sources start consuming the preferred sugar, and then once exhausted, a transcriptional reprogramming trigger the consumption of the less-preferred carbon source (diauxic-shift). During the transcriptional reprogramming, the cells stop dividing, leading to a lag-phase during the microbial growth. Although this represents a well-studied phenomenon, the gene regulatory circuit (GRC) controlling the duration of this lag-phase still remains unveiled. Recent data supports that the HAP transcriptional complex, a stress response regulator, is linked to the lag-phase duration. The lag-phase length is relevant in fermentation, and the *Lager* brewing yeast, a *S. cerevisiae* and *S. eubayanus* hybrid, possesses short or non-existent diauxic lag-phase during fermentations, being its domestication hallmark. The recent isolation of S. eubayanus and the extensive natural diversity of this yeast in Patagonian populations opened new avenues to re-construct and understand the GRC controlling the diauxic lag length on *Lager* yeast. Here, we identify two wild *S. eubayanus* strains exhibiting remarkable opposite diauxic lag-phase duration. Through whole-genome sequencing we recognize genomic changes in HAP complex regulon and glucose-maltose metabolism, which could explain this differential phenotype. Using a functional genomic approach, we generated an episomal luciferase reporter library of candidate promoters of this putative GRC to follow their activity in vivo during diauxic-shift in wild and *knockout* strains for this GRC, the last were made using Crispr-Cas9. With these results we start to re-construct the gene regulatory circuit underlying diauxic lag-phase duration in *S. eubayanus* and unveil the potential genomic changes during *Lager* brewing yeast domestication.

This work was funded by ANID—Millennium Science Initiative Program—Millennium Institute for Integrative Biology (iBio ICN17_022) and ANID/FONDECYT Postdoctorado 3220597.

"dTrmO is required for efficient ACT codon decoding and tissue-specific development in *D. melanogaster*"

Deborah Cuper[1], Jorge Zúñiga-Hernández[1], Valentina Muñoz-Madrid[1], Francisca Brown[1] and Álvaro Glavic[1]

[1] Centro de Regulación del Genoma, Facultad de Ciencias, Universidad de Chile, Las Palmeras 3425, Ñuñoa, Santiago, Chile.

Translation is an extremely fine-tuned process. Both speed and fidelity are required to sustain translational demand and protein homeostasis in cells, traits that depend on several elements, including transfer RNA (tRNA) modifications. Additionally, it has been demonstrated that proliferative and differentiated tissues display differential susceptibilities to deficiencies in certain tRNA modifications. In the present work, we characterize the role of dTrmO, a tRNA-methyltransferase that methylates t^6A in position 37 of tRNAs decoding ACY codons, during *Drosophila melanogaster* development. Our results show that this enzyme is necessary for proper translation of ACT codons, and its function is differentially required in proliferative and non-proliferative tissues. Furthermore, we observed that loss of dTrmO is detrimental for cell growth and proliferation, and muscle function due to impaired translation of Msp300, a nuclear membrane protein specific of muscle fibers. Strikingly, tissue-specific phenotypes produced due to decreased levels of dTrmO in the wing and eye-antenna imaginal disc, fat body, and striated muscles can be reverted by overexpression of $tRNA^{Thr}_{AGT}$. Taken together, our work points the importance of dTrmO in translation of ACT codons and consequently in cell proliferation and growth of tissues, thus providing further insight about the active role of tRNA modifications in the context of eumetazoan development.

Funding: This work was funded by FONDECYT 1190119 (ANID).

"SUMO proteome during macrophage polarization"

Alexei Cuevas[1], Michael Tatham[2], Ronald T. Hay[2], Alejandro Rojas-Fernandez[1].

[1]Institute of Medicine, Universidad Austral de Chile, Valdivia, Chile. 2Centre for Gene Regulation and Expression, College of Life Sciences, University of Dundee, Dundee, United Kingdom.

Inflammation is an adaptive response triggered by adverse stimuli and conditions, which aims to restore homeostasis. This response depends on the coordination of a group of cells belonging to the immune system. One of these cells are the macrophages, which play a role as sensor, coordinator and modulator of the immune response and which have pro- and anti-inflammatory functions. Macrophages grown in a high glucose medium lead to increased production of pro-inflammatory cytokines, resulting in an inappropriate inflammatory process associated with various chronic inflammatory pathologies. Little is known about the role of the post-translational modification SUMO in macrophage polarization. In this work we determine the levels of proteins of the SUMOylation pathway in different stages of macrophages, cultured in medium with 5mM and 15mM glucose. To study of the SUMO proteome in macrophages, we generated THP-1 cells, which express recombinant His-tag SUMO2 using lentivirus. These cells allow us to concentrate the SUMOylated proteins, and by using a mass spectrometry, applying the Label-free Quantification (LFQ) methodology to identify and quantify many proteins. With these data, were determined the differences in the SUMOylation in the different conditions, monocytes vs macrophages M0, and macrophages M1 cultured in 5mM glucose vs. macrophages M1 cultured in 15mM glucose. In this work we present the changes that occur in the SUMOylation of proteins during macrophages polarization the impact of the exposure of high glucose concentrations on the SUMO proteome of THP1 cells differentiate to macrophages.

Funding: This work was funded by FONDECYT REGULAR 1200427 to ARF; FONIS EU-LAC T010047 to CSR, PCC, JO, JB, NLR & ARF; FONDEQUIP EQM180037 to ARF; the regional Council of the "Los Rios region" project FICR16-19, FICR19-20 and FICR21-01;

"Phytochrome A (*DcPHYA*) codes for a functional photoreceptor required for carotenoid synthesis in the carrot taproot grown underground"

Rocío Quian[1], Fabiana Munizaga[1] ,Claudia Stange[1]

[1] Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Las Palmeras 3425, Santiago, Chile

Phytochromes, including PHYA-E are photoreceptors activated by light that participate in vegetative tissue photomorphogenesis (reducing hypocotyl length) and in chlorophyll and carotenoid synthesis. Carotenoids are orange, red and yellow pigments that participate in photosynthesis, among other essential processes. PHYA is active and stable under Far-red (FR) light (shade condition) were it promotes photomorphogenesis and carotenoid synthesis. The orange carrot taproot (Nantes var.) synthetizes and accumulates high level of carotenoids growing underground. Contrary to other plants, white light (WL) impairs both, carotenoid synthesis and root development. To understand the genetic regulation, we performed a RNA-seq between root grown in WL (R/L) and underground (R/D). Genes related to WL signaling, such as DcPHYA are overexpressed in R/D (Arias, et al., 2020). Here we present evidences on the functional characterization of DcPHYA, which exhibits 73% amino acid identity with AtPHYA in their functional domains. Nicotiana tabacum overexpressing *DcPHYA*, present reduced hypocotyl length and reduced expression of *NtPEPCK* and *NtPAR1* under FR, suggesting that DcPHYA is functional. Carrot transgenic lines with over 80% reduced relative expression of *DcPHYA* present between 65%-80% reduction in carotenoid content as well as in the relative expression of *DcPSY1, DcPSY2* and *DcLCYB1* in the taproot grown underground compared to wild-type plant. These results suggest that *DcPHYA* is functional and participates in carotenoid synthesis in the carrot taproot grown underground.

Funding: This research was funded by National Agency for Research and Development (ANID) Fondecyt 1180747 and 1221399 (CS) and the ANID PhDScholarship 2018-21181369 (RQ).

"Exploring the extent of casein kinase C-terminal tail as an autoregulatory domain in circadian mechanisms."

José I. Costa[1, 2], Felipe M. Guzmán[1,2], Luis F. Larrondo[1,2]
Research Communication Sponsor: Luis F. Larrondo[1,2]

[1] Fungal Genetics and Synthetic Biology Lab, Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile
[2] ANID–Millennium Science Initiative–Millennium Institute for Integrative Biology (iBIO), Santiago, Chile.

Casein Kinase 1, CK1, is an essential enyme participating in a plethora of cellular procceses. Importantly, CK1 also plays a pivotal role in all eukaryotic circadian systems described to date. The enzyme can modulate circadian period and is involved in temperature compensation, making of this protein a crucial regulatory hotspot for clock-related inputs. It is hypothesized that regulatory aspects of CK1 general activity rely on the interaction of its unstructured C-terminal tail with the active domain of the kinase. However, such mechanisms in the context of circadian clocks remain unclear. In this work, we explored the regulatory motives of CK1 by dissecting the enzyme structure and analyzing circadian parameters. Using the fungus *Neurospora crassa* as a working model, we made several different CK1 C-terminal tail truncated mutants, and evaluated their effect on clock period using *in vivo* luminescence reporters. We found a 6-hour period shortening in mutants that fully lacked the the third exon, whereas we saw no difference in mutants above this region. By serendipity, we also isolated a mutant carrying a single point mutation on CK1 kinase domain that exerts a clock period delay of 6 hours. Seeking to further characterize the CK1 Kinase domain-tail interaction, we combined the autoregulatory tail mutants with the kinase domain mutant. The effect on clock period seen in combination added to the localization of the mutated site suggests a possible point for intramolecular self-regulation. All together, these results imply that CK1's third exon encodes for an essential autoregulatory structure and may provide yet uncharacterized mechanism to actively modulate period upon certain imput stimuli.

Funding: This work was funded by the ANID-Millennium Science Initiative Program-ICN17_022, ANID-FONDECYT grant numbers 1211715 and ANID-Subdirección de Capital Humano/Doctorado Nacional/2020-21200141.

"Determination of the hemocyanin structure from *Concholepas concholepas* using an X-ray crystallography and Cryo-EM combined approach"

Sebastián M. Muñoz[1], Michelle L. Salazar[2], Gabriel Vallejos[1], Augusto Manubens[2,3] Mathias N. Ellena[4], José Edwin N. Quesñay[4], Andre LB. Ambrosio[4], María Inés Becker[2,3] Víctor Castro-Fernández[1], Victoria Guixé[1*]
* Research Communication Sponsor

[1] Laboratorio de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Chile. Santiago, Chile.
[2] Fundación Ciencia y Tecnología para el Desarrollo (FUCITED). Santiago, Chile.
[3] Laboratorio Investigación y Desarrollo, Biosonda S.A. Santiago, Chile.
[4] Instituto de Física de São Carlos, Universidade de São Paulo. São Paulo, Brasil.

Hemocyanins are giant glycoproteins present in the hemolymph of some mollusks and arthropods. In biomedical and clinical applications, hemocyanins are used as natural, non-toxic, and non-specific immunostimulants. Mollusk hemocyanins present a complex structural organization; the quaternary structure is a didecamer formed by one or two subunits containing eight globular domains called functional units (FU).
Concholepas concholepas hemocyanin (CCH) presents unique structural properties and is highly effective in inducing beneficial immunomodulatory responses. However, despite the biomedical potential of CCH, its structure and amino acid sequence remains unknown. Preliminary biochemical characterization reveals that CCH corresponds to a heterodidecamer conformed by two distinct subunits (CCHA and CCHB). To obtain structural information on CCH, we employed a combined approach: 1) Obtaining a high-resolution structure of an FU by X-ray crystallography, and 2) Docking the FU structure into a density map of the entire hemocyanin obtained by cryo-EM.
We purified a wild-type FU from the CCHB subunit whose crystallographic structure was determined at atomic resolution (1.5 Å). This approach allows us to obtain the sequence and identify the FU as CCHB-g. Also, we determined an N-glycosylation tree with unusual fucose monomers and analyzed the chemical interactions at the copper-binding center. Finally, we determined the first subunit organization model of a heterodidecameric hemocyanin using a near-atomic resolution cryo-EM map of CCH (3.2 Å) and the FU crystallographic structure.
The structural information obtained in this work will be essential for future applications of CCH. In addition, it will contribute to the structural study of mollusk hemocyanins.

Funding: This work was supported by FAPESP/CONICYT 2019/133 (VG), FONDECYT 1201600 (MIB), FONDECYT 1191321 (VG) and FONDECYT 1221667 (VCF).

"Catalytic thermoadaptation in the ADP-dependent sugar kinase family is not driven by changes in the heat capacity of activation"

Ignacio Aravena-Valenzuela[1], Pablo Maturana[1], Felipe González-Ordenes[1], Gabriel Vallejos-Bacceliere[1], Víctor Castro-Fernández[1], Victoria Guixé[1]

[1] Laboratorio de Bioquímica y Biología Molecular, Departamento de Biología, Facultad de Ciencias, Santiago, Chile

Life has adapted to niches with extremely different temperatures, and enzymes from different organisms have had to contend with this evolutionary pressure by modulating their stability and activity. As temperature rises, enzymatic activity increases upon reaching a maximum and then decreases, which has been commonly assumed to be caused by denaturation. However, the macromolecular rate theory (MMRT) claimed that changes in the heat capacity of activation, $\Delta C_P^{\ddagger}$, are essential for explaining the temperature dependence of enzyme-catalyzed reaction rates which involves a temperature dependence of the thermodynamic activation parameters $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$. To address the relevance of changes in these thermodynamic parameters for the catalytic thermoadaptation of enzymes from the ADP-dependent sugar kinase family from archaea, we perform substrate saturation curves at different temperatures of a psychrophilic and a mesophilic enzyme employing the MMRT equation. For both enzymes, plots of the k_{cat} dependence with temperature display two regimens: from low to mild temperatures, they have a $\Delta C_P^{\ddagger}$ in the range of ±2 kJ/mol/K, while at high temperatures the dependence of the $\Delta H\ddagger$ and $\Delta S^{\ddagger}$ values with temperature change dramatically, showing an extremely negative $\Delta CP\ddagger$ which correlates with a change in the secondary structure content. Furthermore, a mutant of the psychrophilic enzyme with altered conformational dynamics that resembles that of the mesophilic enzyme lost the second regimen and a $\Delta C_P^{\ddagger}$ of -1.1 kJ/mol/K was obtained.

Further studies are needed to unravel the determinants and significance of the second regimen at high temperatures.

Funding: FONDECYT N° 1191321

"Lost in Translation: Interfering with the Folding of Nascent Pili Proteins"

J Andrés Rivas Pardo, Tomás Hermosilla, Vicente Colarte, Ivana Orellana, Marcelo J orquera

Mechano-Biology Group, Microbe Genomics Lab, Center for Genomics and Bioinformatics, Universidad Mayor, Santiago, Chile

Gram-positive bacteria use their adhesive pili to attach to host cells during the early stages of a bacterial infection. These extracellular hair-like appendages experience mechanical stresses of hundreds of picoNewtons; however, the presence of an internal isopeptide bond prevents the protein from unfolding. By combining peptide computer-based design, protein engineering, and single-molecule force spectroscopy, we aimed to implement a peptide-based strategy to interfere with the folding of Streptococcal pili proteins.

We developed a python-based open software that allows us to generate a library of peptides and determine the theoretical energy of binding through molecular docking. These results are supported later with molecular dynamics experiments, which together enable us to choose the best peptide candidate for interfering with the folding of pilin proteins. After selecting the peptides, we used protein engineering to add Tags for easy detection and immobilization. Finally, biochemical and single-molecule mechanical assays evaluate the effectiveness of blocking the folding of the streptococcal pili proteins.

Our results show that our strategy for designing engineered peptides yields molecules that compete with the formation of the isopeptide bond on nascent pili proteins, interfering in the proper folding of molecules. While intact pilin molecules are inextensible when mechanical forces are applied, proteins treated with our isopeptide-blocker peptides become fully extensible. We propose that this isopeptide-blocker peptide affords a novel strategy for mechanically-targeted antibiotics, which, by blocking the folding structure of bacterial pili, could prevent the colonization of infectious microorganisms.

Funding: This work was supported by the National Agency for Research and Development (ANID), FONDECYT 1221064, and FDP PEP I-2022017 & I-2022009 UMayor.

"How does one enzyme activity emerge and another disappear during the evolution of an enzyme family? Biochemical and evolutionary analysis of the ATP-dependent vitamin kinase family"

Nicolás Fuentes-Ugarte[1], Myriam Pérez[1], Isaac Cortes[1] ,Gabriel Vallejos[1], Victoria Guixé[1] Víctor Castro-Fernández[1]

[1] Laboratorio de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Chile, Santiago, CL

In the ATP-dependent vitamin kinase family, two homologous enzymes can perform the phosphorylation of hydroxymethylpyrimidine phosphate (HMP-P): 1) the hydroxymethyl-pyrimidine phosphate kinase encoded by the *thiD* gene (ThiD-HMPPK) that phosphorylate HMP to hydroxymethyl-pyrimidine-phosphate (HMP-P), and also phosphorylate HMP-P to produce hydroxymethyl-pyrimidine-diphosphate (HMP-PP). 2) A bifunctional pyridoxal kinase encoded by a *pdxK-like-HMPPK* gene (PdxK-PLK/HMPPK) that only phosphorylate HMP to produce HMP-P, besides the phosphorylation of pyridoxal (PLK activity). Interestingly, phylogenetic analyses indicate that PdxK-PLK/HMPPK enzymes diverged from ThiD-HMPPKs, suggesting that the HMP-P kinase activity would have been lost along evolution while the PLK activity would appeared as an evolutionary novelty in the PdxK-PLK/HMPPK group.

We crystallized an ancestral ThiD-HMPPK from the order *Enterobacteriales* (AncEnHMPPK) in complex with HMP-P and non-hydrolyzable analogs of ATP, and identified crucial residues for HMP-P binding and catalysis. To determine how HMP-P phosphorylation evolved towards pyridoxal phosphorylation in this family, we reconstructed ancestral sequences from these enzyme family, analyzed key residues conservation for the HMP-P phosphorylation and traced them through evolution, elucidating the point mutations that would have occurred during the divergence of PdxK-PLK/HMPPK from ThiD-HMPPK enzymes. These analyses indicate that mutations A110C, H179A and T211A (AncEnHMPPK numeration) would have been the main changes at the active site of ThiD-HMPPK during the evolution to PdxK-PLK/HMPPK, showing that the appearance of PLK activity was concomitant with the disappearance of the HMP-P kinase activity.

Funding: Fondecyt 1221667 and 1191321.

"Coevolution-derived non-native contacts are key to correctly fold RfaH"

Pablo Galaz-Davison[1,2], Diego U. Ferreiro[3], César A. Ramírez-Sarmiento[1,2]

[1] Institute for Biological and Medical Engineering, Pontificia Universidad Católica de Chile, Santiago, Chile.
[2] ANID – Millennium Science Initiative Program – Millennium Institute for Integrative Biology (iBio), Santiago, Chile.
[3] Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina.

The NusG protein family is structurally and functionally conserved through all domains of life, as they directly bind to RNA polymerases to regulate transcription. The RfaH subfamily displays distinct features than NusG, as they regulate expression of virulence factors in a sequence-dependent manner. This is achieved through a structural interconversion between an active fold, the canonical NusG architecture, and an autoinhibited, novel fold. This process is called fold-switching, and proteins displaying it are classified as metamorphic, but how this autoinhibited state is stabilized during evolution is unknown. In this work, we used deposited genomic and metagenomic sequences through Hidden Markov Models to construct a complete RfaH multiple sequence alignment. We further filtered these sequences by predicting their metamorphic features at the secondary structure level using JPred. Coevolutionary signals were then inferred through DCA or Gremlin, and these signals were used to generate structure-based models for molecular dynamics (MD) to predict the structures of RfaH via simulated annealing.
Using this approach, we show that a metagenomic enrichment and filtering protocol increases the chance of correctly identifying physical contacts of the autoinhibited RfaH fold. MD simulations using these contacts led to structures of autoinhibited and active RfaH with RMSD values ~5Å, and simulations of subsets of these residue pairs show that non-native contacts play a key role in achieving the autoinhibited RfaH fold. These results demonstrate that coevolutionary signals found in RfaH sequences encode the autoinhibited and active folds of this protein, shedding light on key interactions in this metamorphic protein.

Funding: FONDECYT 1201684, ANID Millennium Science Initiative Program ICN17_022, ANID PFCHA 21181705

"Evolutive trajectory of the AMP activation in ADP-dependent sugar kinases from methanogenic archaea"

Sixto M. Herrera[1], Gabriel Vallejos[1], Víctor Castro-Fernández[1], Victoria Guixé[1]

[1] Laboratorio de Bioquimica y Biologia Molecular Laboratory/Center,
Department, Faculty, University, City, Country

Unlike the canonical Embden – Meyerhof pathway, in archaea from the Phyla Euryarchaeota, the Glucokinase (GK) and Phosphofructokinase (PFK) activities are carried out by ADP-dependent enzymes. These reactions constitute important allosteric regulation points in the canonical glycolysis, however, in the ADP-dependent kinases of Archaea, this regulation is absent. Nonetheless, AMP activation in bifunctional ADP-dependent PFK/GK enzymes belonging to methanogenic microorganisms from the orders *Methanococcales* and *Methanosarcinales*, has been reported recently.

This work describes the evolutive trajectory of the AMP activation in the ADP-dependent sugar kinases family from Euryarchaeota. Ancestral bifunctional enzymes from the orders *Methanococcales* and *Methanosarcinales*, display AMP activation being a basal and conserved trait within the lineage of bifunctional enzymes. The activation mechanism of ancestral bifunctional enzymes was determined, and results to be different between the ancestors and to the one determined for the extant bifunctional GK/PFK enzyme from *M. maripaludis*. Furthermore, through docking assays and molecular dynamics, we were able to propose a putative allosteric site, validated by bioinformatic assays.

Funding: This work was supported by Fondecyt (1191321)

"A membrane fusion trigger for Halorubrum pleomorphic virus-6 in haloarchaeal cells"

Eduardo A Bignon [1, 2], Kevin R Chou [1], Elina Roine [3], Nicole D Tischler [1, 2]

[1] Laboratorio de Virología Molecular, Centro Ciencia & Vida, Fundación Ciencia & Vida., Santiago, Chile
[2] Facultad de Medicina y Ciencia, Universidad San Sebastián., Santiago, Chile
[3] Molecular and Integrative Biosciences Research Program, University of Helsinki., Helsinki, Finland

Membrane fusion has been extensively studied in eukaryotic cells and their viruses but little information is yet available in the archaea domain. We recently shown that infection by halorubrum pleomorphic virus-6 (HRPV-6), a lipid enveloped virus infecting haloarchaea, involves virus-cell membrane fusion which is driven by the viral VP5 spike protein. Here we aimed to identify the host cell factor that triggers HRPV-6 cell membrane fusion and therefore analyzed the role of the S-layer from its natural host *Halorubrum spp. SS7-4.* To approach this aim we established an S-layer extract-proteoliposome system under native conditions. The depletion of the host cell S-layer abrogated HRPV-6 membrane fusion, suggesting the lack of the physiological trigger. The principal component of the S-layer extract obtained by Mg^{2+} chelating was a ~100 kDa protein identified by mass spectroscopy as the predicted S-layer protein of *Halorubrum spp. SS7-4*. When we reconstituted the S-layer extract onto liposomes, HRPV-6 membrane fusion could be readily observed with proteoliposomes highly enriched in S-layer. This was not the case with liposomes alone or with proteoliposomes carrying the S-layer extract from another haloarchaea not susceptible to HRPV-6, such as *Haloferax volcanii*. Together, we have developed a virus-receptor system in vitro for diverse archaea that allows for the identification of minimal components of membrane fusion, and which suggests that a component of the S-layer extract of *Halorubrum spp. SS7-4* corresponds to the physiological fusion trigger of HRPV-6.

Funding: Volkswagen Foundation (Germany) and ANID (Chile) grants Centro Ciencia & Vida BASAL FB210008 (Excellence Center) and FONDECYT postdoctoral 3190415.

"Sonic hedgehog (Shh) and Trefoil factor 3 (Tff3) are useful biomarkers to study precancerous lesions related to the gastric carcinogenesis"

Ana Vargas[1,2,4] , Francisca Vidal[1,2,4] , Roxana González-Stegmaier[1], Karina Cereceda[1] Constanza Cárcamo[1], Celia Podesta[3], Carlos Barrientos[3], Franz Villarroel-Espíndola[1]

[1] Gastrointestinal Immuno-oncology group, Translational Medicine Research Laboratory. Instituto Oncológico Fundación Arturo López Pérez (FALP), Santiago, Chile.
[2] School of Medical Technology, Faculty of Medicine and Science. San Sebastian University. Santiago, Chile.
[3] Endoscopy Unit. Instituto Oncológico Fundación Arturo López Pérez (FALP), Santiago, Chile.
[4] Contributed equally

Gastric carcinogenesis (GC) is a multistep and multifactorial process and is the result of the interplay between genetic susceptibility and environmental factors. GC involves, in most cases, a progression from normal mucosa through chronic gastritis, mucosal atrophy and intestinal metaplasia to dysplasia and carcinoma. The two diagnostic tools Operative Link on Gastritis Assessment (OLGA) and on Gastric Intestinal Metaplasia (OLGIM) are based on histological assessment for the identification of precancerous lesions. Shh shows a cytoprotective role in gastrointestinal tissue and Tff3 is expressed by the intestinal caliciform glands. We hypothesized that those proteins will correlate with gastric atrophy and metaplasia, respectively. In this work, we evaluated the simultaneous detection of Shh, Tff3 and E-cadherin using multiplexed immunofluorescence (mIF) as an objective and reliable strategy for phenotyping and quantifying gastric precancerous lesions in clinical samples. The results for Shh showed a cytoplasmic staining pattern, with homogenous areas and active secretion foci. Tff3 had a cytoplasmic-membranous staining and was exclusively detected in globet cells. As expected, E-cadherin stained the cell membrane of epithelial cells and did not show correlation with precancerous lesions. The supervised digital pathology assessment by QuPath and the pathologist´s diagnosis showed a concordance rate depending on the OLGA/OLGIM score. Cases classified as non-pathological or score 0 showed the lowest concordance (30%), mainly by isolated metaplastic foci detected by mIF. The combination of immunostaining and digital analysis may increase the sensitivity and specificity to study the premalignant cascade of gastric cancer compared with classic methods, improving early diagnosis and treatments.

Funding: This work was supported by The National Fund for Scientific and Technological Development, FONDECYT (N° 1221415 to FVE) and Internal grant from Fundación Arturo López Pérez (FALP-LMT-2022 to FVE).

"Effect of palmitate and L-NAME on vascular smooth muscle cell viability and senescence"

Javiera Martínez Bilbao [1,2], Dr. (c) Fernanda Sanhueza Olivares [1] & Dr. Mario Chiong Lay [1]

[1] Advanced Center for Chronic Diseases, Faculty of Chemical and Pharmaceutical Sciences, University of Chile.
[2] School of Biochemistry, Austral University of Chile, Valdivia, Chile
Contact: javiera.martinez@ciq.uchile.cl

As life expectancy grows globally, the appearance of age-related diseases increases. One of these conditions is heart failure with preserved ejection fraction (HFpEF), which has comorbidities such as hypertension, diabetes, obesity and aging. It has been proposed that one of the main characteristics of HFpEF is vascular dysfunction. We propose that vascular smooth muscle cells (VSMCs) senescence plays a major role in HFpEF-associated vascular dysfunction. Currently, HFpEF doesn't have a pharmacological treatment because, until recently, an animal model was not available. HFpEF mice model consists in the induction of nitrosative stress in the heart by a combination of a high fat diet and L-NAME. Therefore, we evaluated whether the A7r5 VSMCs treated with a mixture of palmitate and L-NAME, that emulates *in vitro* a HFpEF condition, triggers VSMC senescence. Toxicities of L-NAME and palmitate were determined by dose response assays assessing cell viability by cell count, MTT, and PI permeability by flow cytometry. Non-toxic concentrations of L-NAME and palmitate after 72 hours of stimuli were 5 mM and 1 nM respectively. As noted by flow cytometry, treatments result in a change of A7r5 VSMC morphology. Senescence was assessed by evaluating p16, p21, p53 and H2A.X by western blot and by senescence-associated β-gal activity.
Our results suggest that the mixture of L-NAME and palmitate can be used as an *in vitro* model to emulate HFpEF in VSMC. Further work is required to evaluate whether this *in vitro* model can recapitulate all features observed in the *in vivo* HFpEF mice model.

Funding: This work was supported by the Agencia Nacional de Investigación y Desarrollo (ANID) Chile (FONDECYT 1220392 and FONDAP 15130011).

"Quantitative Proteomic analysis of SUMO modification rearrangements upon starvation: An unknown role for SUMO"

Yorka Cheuquemilla[1], Hector Mancilla[1], Michael Tatham[2], Triim Tammsalu[2], Ronald T. Hay[2], Alejandro Rojas-Fernandez[1].

[1]Institute of Medicine, Universidad Austral de Chile, Valdivia, Chile. [2]Centre for Gene Regulation and Expression, College of Life Sciences, University of Dundee, Dundee, United Kingdom.

The Small Ubiquitin-Like Modifier, SUMO, is an essential covalent post-translational modification in eukaryotes. SUMO can interact non-covalently with proteins based on small hydrophobic patches known as SUMO Interacting Motif (SIM). Therefore, SUMO-modified proteins gain the ability to interact with SIM containing proteins to facilitate the assembly of large complexes such as DNA repair foci and PML nuclear bodies. SUMO modification displays many roles, most of them within the nucleus of cells. SUMO plays an important role in transcriptional regulation, DNA damage response, heat shock stress response, it mediates viral resistant responses, DNA replication and even the replication of epigenetics marks. Here, we have acquired an exceptional proteomic data set by applying a stable isotope labelling with amino acids in cell culture (SILAC) to compare SUMO-modified substrates in control cells and cells 4 hour-starved cells. After SUMO substrate enrichment via pulldown, we identified 408 proteins in which the SUMO modification status varied at least twice after starvation compared to control cells. We described an uncharacterized new group of SUMO substrate identified by SILAC proteomic. This new SUMO substrates, 155 in total, are enhanced by starvation, a lot of them are nucleolar proteins. Finally, we observed an increases number and size of a SUMO 2 nucleolar bodies structure in starved cells compare to the control. Further analysis indicates a dramatic redistribution of SUMO modification upon starvation in cells, likely as an uncharacterized SUMO mediated stress response to starvation.

Funding
This work was funded by FONDECYT REGULAR 1200427 to ARF; FONIS EU-LAC T010047 to CSR, PCC, JO, JB, NLR & ARF; FONDEQUIP EQM180037 to ARF; the regional Council of the "Los Rios region" project FICR16-19, FICR19-20 and FICR21-01;

"Standardization of a female murine model of heart failure with preserved ejection fraction with different L-NAME doses"

Anita Tapia[1,2], Claudia Muñoz[2], María Paz Ocaranza[2], Clara Quiroga[1,2], Sergio Lavandero[2], Hugo Verdejo[1,2], Pablo F. Castro[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. # e-mail address: astapia@uc.cl

Heart failure with preserved ejection fraction (HFpEF) is a highly prevalent disease in older women and people with metabolic syndrome and its pharmacological management has shown to be ineffective, so there is a need to understand the multifactorial physiopathology to improve the efficacy of treatments. Our aim was standardizing of 2-hits model in female mice, because is one of the closest to human HFpEF phenotype, to then to study the subjacent molecular processes involved. To this, 12-week-old C57BL/6N mice (n=12) were fed a high-fat diet (60% kilocal fat) and different L-NAME doses (a NOS inhibitor, 0.5 g/L to 1,5 g/L in water) or a control group (n=12) for 15 weeks, where weekly body weight, glycemia, blood pressure, walking test measurements were performed.
We found that HFpEF 0,5 L-NAME and HFpEF 1,5 L-NAME female mice presented metabolic syndrome, with a significant weight gain ($p<0.0001$) and increase in blood pressure ($p<0.0001$), abnormalities in glucose handling with an increase in the area under the curve of the glycemia test with respect to the control ($p<0.0001$). However, we do not observe changes in the weight of the heart and lung in these two groups with respect to the control condition.
We partially reproduce the HFpEF model in female mice, Nevertheless, pulmonary congestion and hypertrophy have yet to be demonstrated. Echocardiographic data are also necessary to demonstrate preserved ejection fraction and diastolic dysfunction. When these data it can be determined we will study in detail the molecular pathways and possible treatments of the disease.

Funding: This work was supported by the FONDAP-ACCDiS (15130011) and FONDECYT (1211270, 1200490, 3210469).

"Construction of Bacterial Display Nanobody Libraries using the uLoop system"

Bárbara Kunstmann[1], Niklas Schomisch[1], Fernán Federicci[2], Guillermo Valenzuela-Nieto[1], Alejandro Rojas [1&3]

[1]Institute of Medicine, Universidad Austral de Chile, Valdivia, Chile
[2]Institute for Biological and Medical Engineering, Schools of Engineering, Medicine and Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, Chile.
[3]Berking Biotechnology, Valdivia, Chile

South America is the habitant of native camelids such as alpacas (*Vicugna pacos*) which produce – like rest of the camelidae family - heavy chain only antibodies (HCAb) containing IgG2 & IgG3. From these HCAb, we generated nanobodies by isolating their VHH region that contains three hypervariable regions recognizing antigens. As a result, specific molecules of 14 kDa that are more stable than conventional binders and can be efficiently produced in prokaryotic expression systems. The bacterial display DNA library method here presented, uses the cDNA produced from the extracted RNA from alpaca's B-lymphocytes. For this, the cDNA is classically amplified and incorporated into an acceptor vector. The insert is linked to a surface protein of the bacterium. However, since the standard production of the library requires a lot of time due to digestion and purification steps, we have switched to the uLoop system. This system is based on type IIS enzymes that allow simultaneous digestion and ligation of insert and vector in one step. This kind of restriction enzymes minimizes the autoligation of the vectors, making the process more time and cost-efficient. Finally, we have been able to create a nanobody library with a complexity of $2*10^6$ clones in a few days, ready for clone isolation steps.

Funding
This work was funded by FONDECYT REGULAR 1200427 to ARF; FONIS EU-LAC T010047 to CSR, PCC, JO, JB, NLR & ARF; FONDEQUIP EQM180037 to ARF; the regional Council of the "Los Rios region" project FICR16-19, FICR19-20 and FICR21-01;

An alpaca-derived nanobody protects from lethal Beta and Omicron SARS-CoV-2 infection

Guillermo Valenzuela-Nieto[7], Naphak Modhiran[1,2] , Simon Malte Lauer[3], Alberto A Amarilla[1], Peter Hewins[4], Sara Irene Lopes van den Broek[5], Yu Shang Low[1], Nazia Thakur[1], Benjamin Liang[1], James Jng[1], Devina Paramitha[1], Ariel Isaacs[1], Julian de Sng[1] David Song[4], Jesper Tranekjær Jørgensen[8,9], Yorka Cheuquemilla[7], Jörg Bürger[3], Ida Vang Andersen[4,8], Johanna Himelreichs[7], Christian Spahn[3], Ronald Jara[7], Thorsten Mielke[3] Ronan Macloughlin[10], Zaray Miranda-Chacon[7], Pedro Chana-Cuevas[11], Vasko Kramer[12] Alexander Khromykh[1], Martina Jones[2], Keith Chappell[1,2] , Dalan Bailey[7], Andreas Kjaer[8,9] Matthias Manfred Herth[5,8], Jurado Ann Kellie[4], David Schwefel[3], Daniel Watterson[1,2] , Alejandro Rojas-Fernandez[6,9,11]

[1] School of Chemistry and Molecular Bioscience, the University of Queensland; Brisbane, Australia
[2] Australian Institute for Bioengineering and Nanotechnology; Brisbane, Australia
[3] Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Institute of Medical Physics and Biophysics; Berlin, Germany
[4] Department of Microbiology, University of Pennsylvania; Philadelphia, PA, USA
[5] Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen; Jagtvej 160, 2100 Copenhagen, Denmark
[6] The Pirbright Institute; Pirbright, United Kingdom
[7] Institute of Medicine, Faculty of Medicine & Center for Interdisciplinary Studies on the Nervous System, CISNE, Universidad Austral de Chile; Valdivia, Chile.
[8] Department of Clinical Physiology, Nuclear Medicine & PET, Copenhagen University Hospital, Rigshospitalet; Blegdamsvej 9, 2100 Copenhagen, Denmark
[9] Cluster for Molecular Imaging, Department of Biomedical Sciences, University of Copenhagen; Blegdamsvej 3, 2200 Copenhagen, Denmark
[10] Research and Development, Science and Emerging Technologies, Aerogen Limited; Galway Business Park, H91 HE94 Galway, Ireland
[11] Institute of Philosophy and Complexity Sciences; Santiago, Chile.

Since emergence in late 2019, the highly transmissible coronavirus—severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)—has infected more than 300 million individuals and despite the rapid development of vaccine strategies and preventive, viral transmission persists, and viral variants, such as Omicron variant sub-lineages, with altered virulence and antigenic sites have emerged in successive waves across the globe. Accordingly, there is need for antiviral agents that can target new SARS-CoV-2 variants. Camelid nanobodies are an attractive alternative to human antibodies due to their higher stability and ease of large-scale production. Here, we describe the isolation and

characterization of the RBD-specific nanobody W25, that show superior neutralization activity towards Omicron lineage BA.1 and other SARS-CoV-2 variants. Structure analysis of W25 in complex with the SARS-CoV-2 spike surface glycoprotein rationalizes these observations and pinpoints a novel RBD epitope not covered by the current neutralizing antibody repertoire. We furthermore show that W25 also binds the spike protein from the emerging, more infectious Omicron BA.2 lineage with picomolar affinity. In vivo evaluation of W25 efficacy in a mouse SARS-CoV2 infection model, together with W25 biodistribution analysis in mice, demonstrates very favorable pre-clinical properties. Together, these data demonstrate its potential as antiviral agent and recommend W25 nanobody as a prominent candidate for further clinical development.

Funding:
This work was funded by FONDECYT REGULAR 1200427 to ARF; FONIS EU-LAC T010047 to CSR, PCC, JO, JB, NLR & ARF; FONDEQUIP EQM180037 to ARF; the regional Council of the “Los Rios region” project FICR16-19, FICR19-20 and FICR21-01;

"Search of new sex-related biomarkers to study cardiac hypertrophy: A bioinformatic approach"

Jose Miguel Ulloa[1,2], Matias Guerra[1,2], **Ingrid Oyarzún[1,2]**, Georthan Mancilla[1,2], Pablo Castro[1,2], Hugo Verdejo[1,2], Clara Quiroga[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. # e-mail address: clquiroga@uc.cl

Left ventricular hypertrophy is the most common myocardial structural abnormality associated with Heart failure (HF) and other CVD. Some of them are characterized by a difference in prevalence between women and men. Bioinformatics is a growing way to address these sex differences in CVD providing efficient implementation of advanced computational algorithms to optimize time spent, cost, and accuracy in biological data science. Through a bioinformatic strategy, we asked if there are sex-dependent modulators expressed in cardiac hypertrophy that could be detected and then validated in a primary culture of sexed cardiomyocytes.
Using RNA-Seq transcriptomic data obtained from SRA (PRJNA493624), left ventricular samples from 12 mice knock-out to glucocorticoid receptor were analyzed (50% female and 50% male). This model was chosen because it showed progression to HF in a sex-dependent manner. Through different bioinformatic approaches, we found the overexpression of RGS6 (padj= 5,61E-87, log2FoldChange=0,81), GLP1R (padj= 1,25E-87, log2FoldChange= 2,09), NPPA (padj= 0,0, log2FoldChange= 2,92) and NPPB (padj= 0,0 y log2FoldChange= 1,17) in male, but not in female. To validate our results, we treated sexed rat ventricular cardiomyocytes with the pro-hypertrophic stimulus Norepinephrine (10 nM) for 24 and 48h. Our results showed that NE treatment increased βMHC protein levels only in males, while in gene expression we observed an increase of genes predicted with exception of RGS6 in male and female cells. Although we did not observe a sexual dimorphism in the expression of genes proposed, we observed differences in the response magnitude. It remains to be determined whether these differences may be responsible for those differences observed in the CVD development between women and men.

This work was supported by the FONDAP-ACCDiS (15130011) and FONDECYT (1211270).

"Glutamate receptor-like proteins involvement in stimulus-specific electrical signaling in the moss Physcomitrella patens"

Oscar Arrey-Salas[1], Alex San Martín-Davison[1] , Mónica Yánez[1], Claudio C Ramírez[2,3] Erwan Michard[1]

[1] Laboratory of Plant Cell Environment Sensing and Signalling (PCESS Lab), Instituto de Ciencias Biológicas, Universidad de Talca, Talca 3460000, Chile
[2] Laboratorio de Interacciones Insecto-Planta, Instituto de Ciencias Biológicas, Universidad de Talca, Talca, Chile
[3] Centre for Molecular and Functional Ecology in Agroecosystems, Universidad de Talca, Campus Talca, 3460000, Chile

Stimuli-specific and long-distance signals are critical for plant response to a variety of stressful conditions that they are commonly exposed to (temperature, wounding, salt stress, drought). Both electrical signaling and calcium concentrations waves constitute such propagating signals that trigger systemic responses to environmental conditions. Interestingly, some aspects of the molecular mechanisms of those propagating signals are conserved among eukaryotes. In particular, ionotropic glutamate receptors (Glutamate Receptor-Like Proteins – GLR) have been identified as essential in the propagation of both the neuronal electric signal and its equivalent in plants. The molecular mechanisms of GLRs involvement in plant electrical signal propagation is still unclear. The moss Physcomitrium patens is a suitable model organism to study the evolution of signaling pathways, given the simplicity of structures compared to higher plants. In this moss genome, there are two GLR genes, in contrast to the Arabidopsis thaliana AtGLR family with 20 genes. Here, we evaluated the role of GLRs in electrical signal in moss, measuring variation in voltage against the application of various stimuli in combination with pharmacological treatments targeting GLRs. Subsequently, we contrasted the pharmacological response to measurements in glr1 and glr/glr2 genetic backgrounds mutated for one or two PpGLR genes. Results of both set of experiments will be presented and we will discuss the contribution of GLR in electrical signals in moss.

Funding: This work was supported by Fondecyt Regular1210920.

"Assessment of the methylation index in *Arabidopsis thaliana*"

Ariel Orellana, Álvaro Miquel, Hernán Salinas, Ricardo Yusta

[1] Centro de Biotecnología Vegetal, Facultad de Ciencias de la Vida, Universidad Andrés Bello;
[2] Millennium Institute Center for Genome Regulation (CGR); Santiago, Chile

Methylation of DNA and histones has profound effects on the epigenetic regulation of plants. In addition, methylation of polysaccharides, such as pectin and hemicelluloses, is essential for assembling the plant cell wall. S-adenosylmethionine (SAM) is the methyl donor for the methylation of nucleic acids, proteins, polysaccharides, lipids, lignin and is also involved in the synthesis of ethylene and polyamines; thus, SAM plays a critical role in plants.
One of the products of the methylation reactions is S-adenosylhomocysteine (SAH); which acts as an inhibitor of methylation reactions; thus, methylation reactions are regulated by the concentration of the methyl donor (SAM) and the concentration of the inhibitor (SAH). Hence the SAM/SAH ratio has become known as the methylation index. However, little is known about how this index is regulated in plants. Arabidopsis thaliana is a model plant that became a valuable tool for genetic analysis. Arabidopsis has a low mass, a fully developed plant weighs less than 1 g, and different organs weigh a few milligrams; therefore, highly sensitive assays are required to work with a low amount of material. In order to develop a quantitative, highly sensitive assay to assess the methylation index in Arabidopsis, we develop a UPLC-MS assay to measure SAM and SAH that allows us to analyze individual organs from a single plant. Differences in SAM content were detected among different organs; however, the methylation index remains relatively constant. Despite this, some changes were observed between day and night. Finally, a valuable tool to assess the methylation index has been developed, which should help us to identify genetic and environmental cues that affect methylation in plants.

Funding: This study was generously funded by Fondecyt 1190695 and ANID – Millennium Science Initiative Program – ICN2021_044

"Natural Compounds with an agonist and antagonist role in potassium channels"

Marcelo Ibañez[1,2], Javiera Baeza[1], Dr. David Ramírez[2], Dra. Wendy González[1,3]

[1] Centro de Bioinformática, Simulación y Modelado, Facultad de Ingeniería, Universidad de Talca, Talca, Chile.
[2] Ramírez Lab, Departamento de Farmacología, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile.
[3] Millennium Nucleus of Ion Channel Associated Diseases (MiNICAD), Chile.

Abstract: Potassium (K^+) channels are transmembrane proteins that allow the selective flow of K^+ across the membrane. Many of these channels are expressed in various tissues, playing fundamental roles in the proper functioning of the organism. The failure of these channels can trigger various pathologies. Two separate investigations have been able to model two pharmacophores that have a direct relationship with the activation and blocking of K^+ channels. The antagonist pharmacophore model applies to compounds that modulate TASK-1 and Kv1.5 channels (related with atrial fibrillation) and the other pharmacophore model belongs to agonist molecules of TREK-2, hERG and BKCa channels. The agonist compounds could be used in diseases such as stroke (ischemia), epilepsy and pain promoting the simultaneous opening of neuroprotective channels such as BKCa and TREK-2.
Natural compounds (NC) are chemical molecules from animals or plants that can be found in free and commercial databases. They are an important source for drug development, in their natural conformation or as lead compounds for the development of new drugs. We proposed that, by using a pharmacophore-based virtual screening and molecular dynamics simulations, it will be possible to find natural compounds with an antagonist role (in TASK-1 and Kv1.5 channels) or an agonist role (in TREK2, hERG and BKCa channels). These selected compounds could be relevant in the development of new drugs against atrial fibrillation, stroke (ischemia), epilepsy or pain.

Funding: This work was supported by Fondo Nacional de Desarrollo Científico y Tecnológico (Fondecyt N° 1191133).

"Identification of common structural patterns of PLpro from SARS-CoV and SARS-CoV2 relevant for binding inhibitors by using molecular dynamics simulations."

Claudio Illanes[1] , Julio Caballero[1], José Luis Velázquez-Libera[1], Luis Castillo[1]

[1] Center for Bioinformatics, Simulation, and Molecular; Universidad de Talca; 2 Norte 685; Talca-Chile.

Zoonotic coronavirus (CoV) infections pose a great threat to humans, as they can adapt to new environments through mutation and genetic recombination. The COVID-19 pandemic has indicated the lethality this type of pathogen can achieve. The primary research aims to directly attack the virus and interrupt critical steps of the viral mechanism. PLpro, one of the two proteases responsible for coronavirus replication, performs the cleavage of non-structural proteins and deubiquitination, which is essential for virus infection. In this work, we proposed studying the interactions of nine structurally diverse inhibitors with PLpro by using molecular simulation methods. Firstly, molecular docking calculations were performed, providing the orientations of the inhibitors in SARS-CoV and SARS-CoV2 PLpro binding sites. Subsequently, accelerated molecular dynamics calculations were performed to obtain different protein conformations and sampling of protein-ligand interactions. Finally, similar conformational states of all the PLpro-ligand systems were grouped by clustering. This protocol allowed us to find similar structural patterns associated with SARS-CoV and SARS-CoV-2 PLpro binding sites that determine interactions with inhibitors. We also provided a detailed list of the residues involved in the flexibility of SARS-CoV and SARS-CoV2 PLpro binding sites, which could significantly impact the interactions with inhibitors. The results of this study could be helpful for the rational design of inhibitors against PLpro from future CoVs.

Funding: The assistance to the Annual Meeting of the Society for Biochemistry and Molecular Biology of Chile 2022 was supported by the Fondo Nacional de Desarrollo Cientifico y Tecnologico (FONDECYT) [1210138]. The web server is hosted in the cluster obtained with the grant [CONICYT-FONDEQUIP-EQM160063].

"New protocols to model protein flexibility and identify correct ligand binding poses in in silico docking experiments."

Sergio Alfaro[1], Francisco Adasme [1,2], José Luis Velázquez [1], Fabián Gonzalez [1]
Julio Caballero [1]

[1] Center for Bioinformatics, Simulations and Modelling, Universidad de Talca, Talca 3460000, Chile.
[2] Research Center for Advanced Studies of the Maule, Vice-Rector for Research and Postgraduate Studies, Universidad Católica del Maule, Talca, 3460000, Chile.

Understanding the mechanism of molecular recognition between ligands and proteins is essential in biochemistry and biophysics applied to the rational design of drugs and the creation of new therapies. For this, docking methods have allowed an interpretation of how the ligands recognize their protein targets. Docking methods also yield predicted energies, which ideally can be correlated with the experimental values of affinity/inhibition. However, it is not common to obtain good correlations, and selecting the ideal ligand binding pose with a specific protein conformation remains a significant problem because of the ample search space. In this work, a docking protocol was reported to address these problems. Protein structures of P38 extracted from PDB and conformations of Beta-thrombin generated by gaussian accelerated molecular dynamics (GaMD) were used as receptors in docking calculations. Series of congeneric ligands for each protein were cross-docked to obtain the poses inside the available conformations. A genetic algorithm was used as a search engine to find those ligand binding poses and specific protein conformations that maximize the value of linear correlation between the experimental activities with the predicted energies. The results show that the use of the genetic algorithm allowed us to find a combination of ligand poses and specific conformations of proteins that maximize the linear correlation value between the experimental activity and the predicted energy for both P38 and Beta-thrombin. The protocol could help understand the flexibility of protein binding sites during molecular recognition.

Funding: The assistance to the Annual Meeting of the Society for Biochemistry and Molecular Biology of Chile 2022 was supported by the Fondo Nacional de Desarrollo Cientifico y Tecnologico (FONDECYT) [1210138]. The web server is hosted in the cluster obtained with the grant [CONICYT-FONDEQUIP-EQM160063]

"Binding site comparison from K_v1.5 and Na_v1.5 cardiac ion channels: a computational study with flecainide and AVE0118 antiarrhythmic drugs"

Yuliet Mazola Reyes[1], José Carlos Estanislao Márquez Montesinos[1], Wendy González Díaz [1,2]

[1] Center for Bioinformatics, Simulation and Modeling (CBSM), Universidad de Talca, Chile.
[2] Millennium Nucleus of Ion Channels-Associated Diseases (MiNICAD), Chile.

Introduction: Most antiarrhythmic drugs to treat atrial fibrillation (AF) exhibit multi-target action on ion channels. However, the structural basis of their promiscuity is not well-understood. Here we compared the binding sites (BS) of two antiarrhythmic drugs, flecainide and AVE0118, in potassium K_v1.5 and sodium Na_v1.5 ion channels. The latter are involved in cardiac action potential and constitute relevant targets for AF. Methods: We performed molecular docking to predict flecainide and AVE0118 BS in K_v1.5 and Na_v1.5, respectively. BS comparisons were done using an in-house workflow combining molecular dynamics, BS characterization, and pattern matching. Ala-mutations in Na_v1.5 wild-type were obtained by restriction enzyme cloning and their biophysical characterization by whole-cell patch clamp. Results and Discussion: From docking analysis, we predicted that flecainide and AVE0118 drugs occupy the central cavity and extend to fenestrations in respective K_v1.5 and Na_v1.5 channels. The comparison of BS for AVE0118 is pending to complete Ala-mutations in Na_v1.5 to validate our docking predictions; at present we confirmed F1465A mutation by DNA sequencing. Regarding flecainide, previous structural and mutagenesis data coupled with our docking were sufficient to identify a common structural pattern in Na_v1.5 and K_v1.5. The latter belongs to the central cavity and consists of a hydrophobic patch and a polar region. We also identified a distinctive feature of aromatic residues in flecainide BS in Na_v1.5, probably linked to its higher affinity. Our findings could advance the understanding of drug promiscuity in ion channels, and give insights for multi-target directed ligand design with applications to AF.

Keywords: drug promiscuity, binding site comparison, multi-target, flecainide, AVE0118, Na_v1.5, K_v1.5

Funding: This research was funded by Fondo Nacional de Desarrollo Científico, Tecnológico y de Innovación Tecnológica (FONDECYT) grant number 1191133 and Millennium Nucleus of Ion Channel Associated Diseases (Minicad).

"Docking and Molecular Dynamics Simulations studies of the most abundant toxin of the Blue Scorpion on Nav1.5 ion channel"

Erbio Díaz [1,2], Wendy González [1,2], Vladimir Yarov-Yarovoy [3,4]

[1]Centro de Bioinformática, Simulación y Modelado, Universidad de Talca, Talca, Chile; [2]Millennium Nucleus of Ion Channels-Associated Diseases (MiNICAD), Universidad de Talca, Talca, Chile; 3Department of Physiology and Membrane Biology, University of California Davis, Davis, CA 95616, USA; 4Department of Anesthesiology and Pain Medicine, University of California Davis, Davis, CA 95616, USA.

Voltage-gated sodium channels (Navs) are molecular targets of β-scorpion toxins, which shift the channel activation voltage dependency to more negative membrane potentials by a voltage sensor-trapping mechanism. nNav1.5, the neonatal variant of the most abundant Nav in cardiac tissue, Nav1.5, is over-expressed in MDA-MB-231 breast cancer cell line. nNav1.5 enhances and promotes the invasiveness and progression of cancer cells toward the metastatic stage. nNav1.5 has been proposed as a molecular target to stop breast cancer metastases progression. Blue Scorpion venom has been used in cancer treatment for many years in Cuba. In 2011, the venom was biochemically characterized and the most abundant toxin, RjAa12f, was isolated. RjAa12f is a 64 residue peptide containing four-disulfide bonds. It shares the βαββ-scaffold of the β-scorpion toxins that target Navs. Sequence alignment of homologous peptide toxins revealed similar structural patterns between RjAa12f toxin and anti-insect/anti-mammal neurotoxins. The binding site of the anti-insect/anti-mammal neurotoxins in Navs channels is mainly localized in the S1-S2 and S3-S4 extracellular regions in domain II and in SS2-S6 loop in domain III. We used docking and molecular dynamics simulations studies to characterize the RjAa12f-nNav1.5 complex association based on an extrapolation of the mutagenesis experiments derived from CssIV neurotoxin-rNav1.4/rNav1.2 complexes. MM-GBSA method was used to determine the binding affinity of nNav1.5-RjAa12f complex. Toxin-channel interactions were analyzed using the Protein-Ligand Interaction Profiler (PLIP) interphase. The results suggest important differences between the RjAa12f-hNav1.5 and CssIV-rNav1.4/rNav1.2 complexes. Our study might be useful for the future development of novel peptide-based therapeutics to treat breast cancer.

This work was supported by Fondo Nacional de Desarrollo Científico y Tecnológico (Fondecyt Number 1191133).

Application of bacterial and fungal MAMPs induces Pattern Triggered Immunity in *Prunus persica*

Uri Aceituno-Valenzuela [1], Daniela Muñoz[1], Meirav Leibman-Markus[2], Maya Bar[2], Adi Avni[3] and Lorena Pizarro [1]

[1]Laboratorio de Inmunidad Vegetal. Instituto de Ciencias Agroalimentarias, Animales y Ambientales. Universidad de O'Higgins. San Fernando, Chile.
[2]Plant Pathology and Weed Research, ARO, Volcani Institute, Rishon LeZion, Israel.
[3] School of Plant Sciences and Food Security, Tel-Aviv University, Ramat-Aviv, Israel.

Prunus persica is a highly economically relevant specie from the Rosaceae family. Its cultivation is attacked by several phytopathogens causing severe losses. Plants have an innate immunity system, called Pattern-Triggered Immunity (PTI), that sense conserved Microbe-Associated Molecular Patterns (MAMPs) including peptides, lipids, peptidoglycans and oligogalacturonides. These conserved molecules are recognized by Pattern Recognition Receptors (PRRs), which trigger a signaling cascade and activate defense mechanisms. PTI leads to defense responses, including early reactive oxygen species production (oxidative burst), MAP kinase signaling cascade, transcriptional reprogramming, ethylene production, and, in some cases, programmed cell death. FLS2 and EIX2 PRR receptors had been characterized in arabidopsis and tomato, respectively. FLS2 recognizes flg-22, a peptide from bacterial flagellin from *Pseudomonas spp*, while EIX2 recognizes Xyn11 an ethylene-inducing xylanase from Trichoderma viridie. In this work, we demonstrated that treatment with flg-22 and Xyn11 induce changes in the expression of defense-related genes in four Nectarine cultivars 6 and 24 hrs post application. We identified orthologues of FLS2 and EIX2 in *Prunus persica* through phylogenetic analysis. To determine if the putative receptors are functional, we cloned the ORFs on binary vectors. PRRs were transiently expressed by Agro-infiltration in *Nicotiana benthamiana* and PTI activation mediated by flg-22 and Xyn11 was evaluated by oxidative burst assay. Our results suggest these candidates could be involved in recognizing flg-22 and Xyn11 in Nectarines.

Funding: This work was supported by Fondecyt Iniciación 11200934.

"Co-occurrence Interaction Networks of Bacterial Species Between Two Different Sectors of a Copper Tailings Mine"

Gabriel Gálvez [1], Jaime Ortega [1], Mauricio Latorre [1,2,3]

[1] Bioengineering Laboratory; Institute of Engineering Sciences; O'Higgins University, Rancagua, Chile.
[2] Laboratory of Bioinformatics and Gene Expression, INTA, University of Chile, Santiago, Chile.
[3] Systems biology center for the study of extremophile communities from mining tailings (SYSTEMIX), O'Higgins University, Rancagua, Chile.

Copper mining tailings are characterized by high concentrations of metals and an acidic pH, conditions that require adaptation for any organism. Currently, several bacterial species have been isolated from mining environments; however, little is known about the structure of microbial communities and how their members interact with each other. This work generates a co-occurrence network, representing the bacterial soil community from the Cauquenes tailing, which is the largest copper waste deposit worldwide. A representative sampling of six zones from the Cauquenes tailing was carried out to determine pH, metal concentration, total DNA extraction, and subsequent assignment of Operational Taxonomic Units (OTUs). According to the elemental concentrations and pH, the six zones could be grouped into two sectors: (1) the "new tailing," characterized by neutral pH and low concentration of elements, and (2) the "old tailing," having low pH and a high concentration of metals. Even though the abundance and diversity of species were low in both sectors, the *Pseudomonadaceae* and *Flavobacteriaceae* families were over-represented. Additionally, the OTU identifications allowed us to identify a series of bacterial species with diverse biotechnological potentials, such as copper bioleaching and drought stress alleviation in plants. Using the OTU information as a template, we generated co-occurrence networks for the old and new tailings. The models revealed a rearrangement between the interactions of members living in the old and new tailings, and highlighted conserved bacterial drivers as key nodes. These results provide insights into the structure of the soil bacterial communities growing under extreme environmental conditions.

Funding: ANID FONDECYT 1190742 (ML), ANID BASAL FB210005 (ML), ANID MILENIO ICN2021_044 (ML), ANID ANILLO ACT210004 (ML) and ACT20210044 (ML), Beca ANID 21211367 (GG) and 21220593 (JO).

"Response of antioxidant metabolism on oxidative damage in three phenological stages of potato (*Solanum tuberosum* L.) under different irrigation regimes"

Rodrigo Mora-Sanhueza[1], Paz Cárcamo-Fincheira[2], Ricardo Tighe-Neira[2], Rafael López-Olivari[3], Claudio Inostroza-Blancheteau[2]

[1]Estudiante de Agronomía, Departamento de Ciencias Agropecuarias y Acuícolas, Facultad de Recursos Naturales, Universidad Católica de Temuco, Rudecindo Ortega 02950, Temuco.
[2]Departamento de Ciencias Agropecuarias y Acuícolas, Recursos Naturales, Facultad de Recursos Naturales, Universidad Católica de Temuco, Rudecindo Ortega 02950, Temuco.
[3]Instituto de Investigaciones Agropecuarias, INIA Carillanca, km 10 Camino Cajón-Vilcún s/n, Temuco Casilla Postal 929, Chile.

Water stress in plants, induces overproduction of ROS, as well as, a reduction in the growth and development. Moreover, affects the normal functioning of the cellular processes. Plants have evolved complex biochemical adaptations to adjust and adapt to a variety of stresses. The aim was to evaluate the antioxidant metabolism responses on oxidative-damage in different phenological stages of potato Puyehue-INIA variety with a strategy of deficit-irrigation. Five treatments were applied: (T1; control, 100% ETc), over-irrigation (T2; 30% more than T1), 75% of T1 (T3), 50% T1 (T4), and 30% T1 (T5), using drip-irrigation, replenishing soil water to 35% of total available water (ADT). We evaluated the antioxidant activity (AA), polyphenols (PPh), flavonoids (FV), lipoperoxidation (LP)] in three stages [tuberization (C1), flowering (C2) and fruiting (C3)]. The results showed ($p \leq 0.05$) in the factors: C, T and C x T. In potato leaves the AA increases significantly in C2 and C3 at 1.3, 1.3, 1.2 and 1.4-fold in the treatments T2, T3, T4 and T5, respectively, compared with T1. In the same way, the concentration of PPh increased in C2 and C3 at 1, 1.1, 1.2 and 1.3-folds in the treatments T2, T3, T4 and T5, respectively. FV showed an increase in T3, T4 and T5 ≈1.1, 1.3 and 1.3-folds in C2 and C3. Otherwise, LP increased 1.1, 1.1 and 1.2-fold in T2, T4 and T5, respectively in C2 and C3. In conclusion, it's suggested that the strategy of deficit irrigation provoke an increase in the polyphenol's mitigate water-stress in potato plants.

Keywords: Antioxidant activity, polyphenols, lipoperoxidation, water stress.

Acknowledgment: FONDECYT Iniciación N°1118066 and Regular 1201749.

"Biochemical characterization of an ancestral Phytase from *Enterobacterales* order"

Carlos Montiel[1] , Claudia Mella-Hernández[1], Victoria Guixé[1], Victor Castro-Fernandez[1]

[1] Laboratorio de Bioquímica y Biología Molecular, Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile

Phytases (myo-inositol hexakisphosphate phosphohydrolase) catalyze the stepwise hydrolysis of phytate into myo-inositol and inorganic phosphates. Phytases from *Enterobacterales* belong to the Histidine Acid phosphatases family, and they have been used successfully as a feed additive to improve phosphorus absorption in monogastric animals. Among them, *E. coli* phytase is one of the best characterized enzymes in this family, showing high efficiency and specificity in the hydrolysis of phytate.
In this work, we have performed Ancestral Sequence Reconstruction (ASR) to trace the evolutionary history of the phytase lineages from *Enterobacteriales* (AncEnPhy) to address biochemical properties as the pH optimus, catalytic efficiency,substrate specificity, thermal stability and residues of the active site responsible for phytase activity. AncEnPhy exhibits a molecular weight of 40kDa, maximus acid phosphatase activity between pH 2.0–5.5, a catalytic efficiency lower than exant *E. coli* enzyme and residues of the active site similar to *E. coli* phytase. These results show that the current enzyme of *E. coli* is more specialized for phytate hydrolysis than the ancestral enzyme of the last common ancestor of *Enterobacteriales.*

Funding: This work was supported by the Fondecyt 1221667 and the PhD scholarship of the Facultad de Ciencias from Universidad de Chile.

"Identification and characterization of YAB transcription factors and anthocyanin accumulation under abiotic stress conditions in cultivated tomato (*Solanum lycopersicum* 'Indigo Rose')"

Karla Jara-Cornejo[1,2,3], 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, characteristic domains, and phylogeny. Also, we observed their gene expression profile in different tissues using RT-qPCR, both under normal conditions and under drought and UV-B-associated 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.

Funding:
Beca DOCTORADO NACIONAL/2019 21190862, y DOCTORADO NACIONAL/2020 – 21201520 y 21201418. ANID – Millenium Science Initiative Program – NCN2021_010, Fondecyt 1211180 y 1210941 projects.

"Effect of antinucleosomal DNA sequences on nucleosome remodeling activity of ISW1a and RSC complexes"

Fernanda Raiqueo[1], Roberto Amigo[1], José Gutiérrez[1]

[1] Laboratorio de Regulación Transcripcional, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile.

In chromatin, histones are constantly mobilized so that processes like DNA replication, transcription, or repair can occur. Among the factors that can influence this mobilization, DNA sequence and ATP-dependent chromatin remodeling complexes perform important roles. For example, in gene promoters, the antinucleosomal sequence poly(dA:dT) directs RSC remodeling complex resulting in generation of nucleosome depleted regions, allowing DNA transcription to happen.
Considering that, throughout evolution, different sequences similar to poly(dA:dT) might have developed, we aimed to find out if other sequences were capable of blocking ISW1a's nucleosome sliding activity and to stimulate RSC's sliding activity. By performing chromatin remodeling assays, the effect of [(G/C)3NN]n and (GAA)n sequences was analyzed. Both of them were able to block ISW1a's activity, but neither [(G/C)3NN]n nor (GAA)n were able to stimulate RSC's sliding activity. Interestingly the latter sequence blocked RSC's action. Thus, none of these sequences showed exactly the same effects displayed by poly(dA:dT) sequences. Further assays confirmed that, indeed, (GAA)n inhibits RSC's activity. In addition, we developed a systematic procedure to search for more antinucleosomal sequences, based on competitive reconstitution assays, finding that -when using the 601-nucleosome positioning sequence for these assays -modification of its sequence at SHL+1.5 has the highest impact on nucleosome stability.
Altogether, these findings bring to a better understanding of how defined DNA sequences affect chromatin dynamics. In addition, they can contribute to new studies focused on evolution of DNA sequences at gene regulatory regions and to determine mechanisms by which certain mutations alter normal cellular processes.

Funding: This research was supported by ANID/FONDECYT Regular 1180911.

"Electrostatic amino acid optimization for domain dimerization: magnet concept applied in vivo and in silico to an optogenetic switch"

Leonardo Guzmán-Zamora[1, 3], Sebastián Dehnhardt[2, 3], Vicente Rojas[2, 3], Luis Larrondo[2, 3].

[1]Universidad de Chile, Facultad de Ciencias.
[2]Pontificia Universidad Católica de Chile, Facultad de Ciencias Biológicas.
[3]Instituto de Milenio de Biología Integrativa (iBio).

Photoreceptors are widely used in optogenetic switches since they provide high spatio-temporal modulation of gene expression and use inexpensive resources. One type of photoreceptors are the LOV domains, distinguished by their modular small size and capacity to homo- or hetero-dimerize in response to blue light. The Fungal Light-oxygen-voltage (FUN-LOV) optogenetic transcriptional switch, developed in our lab, is based on the interactions of LOV domains from the fungal proteins WC-1 and VVD. FUN-LOV stands out by its high temporal resolution, prominent level of induction and low background expression.
VVD has already been used to reconstitute the function of split proteins, but it is prone to undesired and non-functional dimerization within same split parts (homo-dimerization). This problem has been solved in the Magnet system by changing amino acids in the dimerization region for electrostatic counterparts, providing selectivity to these switches.
In this work, we conducted a Magnet optimization approach to the FUN-LOV components, by carrying out the amino acid replacement in their corresponding LOV domains. Nevertheless, the original Magnet electrostatic interaction was not recapitulated. However, the system was optimized by negatively charged amino acids in WC-1, while VVD remained in its original neutral form. In fact, in silico characterization suggests an alteration of the dimeric structure in WC-1 by electrostatic repulsion, which could increase the likelihood of VVD interaction, improving the response level. Furthermore, computational predictions including energy changes in the whole protein complex are aiding in the interpretation of this behavior.

Funding by FONDECYT 1211715 and iBio.

"Small non-coding RNA composition of chromatin differs from the nuclear composition in pancreatic adenocarcinoma PANC1 cells"

Michell Martin[1] , Rodrigo Maldonado[1]

[1] Laboratory of RNA and Epigenetics, Instituto de Anatomía, Histología y Patología, Facultad de Medicina, Universidad Austral de Chile, Valdivia, Chile.

Cancer is defined as the rapid proliferation of abnormal cells. This is explained by the deregulation of gene expression leading to altered cellular processes. Currently, regulation of transcription is attributed to non-coding RNA molecules (ncRNAs), among other molecules, through its direct or indirect interaction with chromatin. Among them, a variety of nuclear small ncRNAs, like specific microRNAs (miRNAs), have been described as nuclear components mammalian cells. Several studies have described aberrant changes in miRNA expression profiles in different types of cancer, being the pancreatic adenocarcinoma one of the most affected. Nevertheless, changes on miRNA expression do not always correlate with their canonical cytoplasmic post-transcriptional regulation of mRNA expression. Suggesting another layer of regulation. Therefore, despite being described as nuclear components, miRNAs have not been studied as components of chromatin to explain a putative transcriptional function. In this work, sncRNAs interacting with chromatin and transcriptionally active chromatin were purified and sequenced in pancreatic cancer cells, PANC1, by Chromatin-small RNA ImmunoPrecipitation (ChsRIP). The sequencing results show that the small ncRNAs distribution associated to chromatin is different when compared to nuclear extracts. While chromatin is enriched for miRNAs, the nuclear extract is mainly composed by tRNAs fragments (tRFs). Bioinformatic analysis of our results indicate a low diversity of small RNAs and that overrepresented sequences correspond to 40-50% of sequencing outcome. To our knowledge, these results demonstrate for the first time the miRNA interaction with chromatin, suggesting a putative nuclear function at the transcriptional level, and hence, modulating gene expression in cancer cells

Funding: This work was supported by Comisión Nacional de Investigación Científica y Tecnológica – CONICYT and funded by Fondo Nacional de Desarrollo Científico y Tecnológico – FONDECYT (11190246).

"Identification of non-coding RNAs related to iron homeostasis in *Enterococcus faecalis*"

Sebastián Gomez [1], Jorge Torres [1], Victor Aliaga-Tobar [1], Mauricio Latorre [1,2,3]

[1] Bioengineering Laboratory; Institute of Engineering Sciences; O'Higgins University, Rancagua, Chile.
[2] Laboratory of Bioinformatics and Gene Expression, INTA, University of Chile, Santiago, Chile.
[3] Systems biology center for the study of extremophile communities from mining tailings (SYSTEMIX), O'Higgins University, Rancagua, Chile.

Iron homeostasis in *E. faecalis* is controlled by the regulator Fur. In *E. faecalis* Δfur exposed to basal conditions, a significant change is observed at the global transcription levels. This suggests the presence of other regulatory mechanisms involved in the response of iron homeostasis. Data indicate that non-coding RNA (ncRNAs) are involved in iron homeostasis regulation. That is why the objective of this work is to identify ncRNA regulators of Fe homeostasis and test if its expression is regulated by Fur. Firstly, we update the Fe homeostasis model of *E. faecalis* by increasing the number of components by 36%. Secondly, the search for ncRNAs that were regulating these components of the new *E. faecalis* homeostasis model resulted in 4 ncRNAs. We found that the conservation of these selected ncRNAs occurred in 3 ncRNAs in the *Lactobacillales* order. qPCR data of the selected ncRNAs in the different Fe availabilities revealed that they are capable of changing their abundance, which suggested that must be a transcription factor that modulates this activity and that, in turn, responds to Fe, such as Fur. Quantification of selected ncRNAs in *E. faecalis* Δfur under basal conditions revealed a change in their abundance, which is indicative that Fur could be involved in the regulation of these elements. However, the absence of Fur boxes in the promoters of the ncRNAs suggests an indirect regulation. Finally, these data help to expand the transcriptional network of Fe homeostasis in *E. faecalis* through the identification of regulatory ncRNA of Fe homeostasis systems.

Funding: ANID FONDECYT 1190742 (ML), ANID BASAL FB210005 (ML), ANID MILENIO ICN2021_044 (ML), ANID ANILLO ACT210004 (ML) and ACT20210044 (ML), ANID Postdoctoral 3220080 (VAT).

"In silico characterization of the FaYAB1.1 protein and the relative expression pattern of the FaYAB1.1 gene in different tissues of *Fragaria* × *ananassa*"

Eliana Bustos[1,2,3], Karla Jara-Cornejo[2,3], Paz E. Zúñiga[2,3], Carlos R. Figueroa[2,3]

[1] School of Biochemistry, Institute of Biological Sciences, Universidad de Talca, Talca, Chile.
[2] Laboratory of Plant Molecular Physiology, Institute of Biological Sciences, Universidad de Talca, Chile.
[3] Millenium Nucleus for the Development of Super Adaptable Plants (MN-SAP), Santiago, Chile.

YABs (a.k.a., YABBYs) correspond to a family of transcription factors that play an important role in the growth and development of leaves, flowers, and fruits. In this work, the FaYAB1.1 gene and its different copies present in the octoploid genome of *Fragaria* × *ananassa* were identified and characterized in silico. Sequences were obtained by Blast from the Rosaceae Genome Database (GDR) and their size, ORF, gene structure, and chromosomal location were determined. Also, their promoter regions were analyzed to identify cis-regulatory elements in response to phytohormones [i.e., abscisic acid (ABA), auxin, gibberellin, and jasmonate (JA)]. On the other hand, a multiple alignment was performed with the MEGA software and the YABBY domain was identified using PROSITE. The 3D structure of the FaYAB1.1 protein and its YABBY domain was modeled using MODELLER. FaYAB1.1 expression was also evaluated in different tissues of strawberry (F. × ananassa 'Albion`). According to our results, at least six copies were found for the FaYAB1.1 gene, with regulatory elements for ABA, auxin, and JA in their promoters. The FaYAB1.1 protein presented a structure where alpha-helices, loops, and beta-sheets predominate. A higher expression of FaYAB1.1 was observed in flowers and leaves compared with other tissues. In fruit, gene expression was higher in immature stages. Future studies are expected to analyze the regulation of FaYAB1.1 expression by functional analysis of its promoter.

Funding: This work was supported by the National Research and Development Agency (ANID, Chile) through FONDECYT/Regular 1210941, Millenium Science Initiative Program - NCN2021_010, and BECAS/DOCTORADO NACIONAL/2020 21201520 and 21201418.

"Differential expression of Innexins in insecticide insensitive clones of *Myzus persicae"*

María Eugenia Rubio-Meléndez[1], Claudio Valenzuela[2], Angélica González[3], Ingo Dreyer[1]

[1]Centro de Bioinformática y Simulación Molecular (CBSM), Facultad de Ingeniería, Universidad de Talca, Talca, Chile.
[2]Center for Medical Research, Medical School, University of Talca, Talca 3460000, Chile.
[3]Doctorado en Ciencias con Mención en Biología Vegetal y Biotecnología, Instituto de Ciencias Biológicas, Universidad de Talca, Talca, Chile.

Aphids (Hemiptera: Aphididae) plant sap-feeding stinging-sucking insects with dynamic life cycles, which develop both on their primary host (woody plants) and secondary hosts (herbaceous plants). In addition to their reproductive advantages and economic damage to crops, they are considered pests. In this sense, aphid control has been challenging due to insecticide resistance acquired at the population level; an insensitivity in aphids is passed on from generation to generation. In this sense, searching for new target sites that can be used in new strategies for aphid control has become important. For this purpose, we analyzed the intercellular gap junction (GJ) channels, encoded by the innexin gene family, present in all invertebrate organisms as a possible target for control. By qPCR analysis, the expression of five innexins (Inx1, Inx2, Inx3, Inx7, and Inx-SB) was determined in different females from 6 lineages of *M. persicae* susceptible and insecticide insensitive lineages selected with TaqMan probes that identify the knock-down resistance mutation (*Kdr*), Super-kdr (*Skdr*) and the acetylcholinesterase enzyme mutation S431F (MACE). The results indicate significant differences in the expression of the five genes analyzed as a function of the insensitivity of the clones, where those clones susceptible to MACE tended to have low expression in all genes, while those with high expression in the genes tended to be insensitive to MACE.

Funding: This work was supported by Fondo Nacional de Desarrollo Científico y Tecnológico: Fondecyt Postdoctoral 3190544 (to MER-M), Fondecyt Iniciación 11201182 (to CV) and Fondecyt Regular No. 1150054 and No. 1220504 (to ID).

"Evaluation of ALMT and MATE genes in genotypes of highbush blueberry (*Vaccinium corymbosum* L.) under different doses of Al-toxic and acid conditions"

Paz Cárcamo-Fincheira[1], Ricardo Tighe-Neira[1], Adriano Nunes-Nesi[2], Marjorie Reyes-Díaz[3], **Claudio Inostroza-Blancheteau[1]**

[1]Departamento de Ciencias Agropecuarias y Acuícolas, Facultad de Recursos Naturales, Universidad Católica de Temuco, Rudecindo Ortega 02950, Temuco.
[2]Departamento de Biologia Vegetal, Universidade Federal de Viçosa, 36570-900 Viçosa, MG, Brazil.
[3]Departamento de Ciencias Químicas y Recursos Naturales, Facultad de Ingeniería y Ciencias, Universidad de la Frontera, Francisco Salazar 01145, Temuco.

Plants have developments several strategies to reduce Al-toxicity. These Al-resistant mechanisms can be divided into: Al-exclusion and Al-tolerance. Molecular mechanisms, such as ALMT and MATE genes, could be involved in both strategies. The aim of this study is to evaluate the responses of the ALMT and MATE genes to different concentrations of Al-toxic and acid conditions in three genotypes of blueberry with differential responses to Al3+ [Star (Al-sensitive), Cargo (Al-resistant), and Liberty (undetermined)] subjected to 0 (T1), 200 (T2), 400 (T3) and 800 (T4) µM Al (AlCl3), in Hoagland solution at 4.5 pH during 48-hours. Aluminum content and expression levels of ALMT and MATE in roots and leaves were evaluated. The results showed significant differences ($p \leq 0.05$) in the all factors. Al-content increased in all treatment and genotypes, in roots ~6 to 11-fold in Star, 10 to 20-fold in Cargo, and 4 to 7.5-folds in Liberty, during all times-evaluated. Meanwhile, in leaves of the all genotypes Al-content increased in T4 ~1.3 to 1.6-fold at 24 and 48-h. Expression levels, in roots and leaves ALMT diminished in all genotypes and treatments ~53 to 98% at 24-h, while in Star leaves increased ~1.3 to 1.6-folds in all treatment at 48 h. In roots and leaves of all genotypes MATE showed a decrease ~45 to 82% in both treatment T2 and T4 at 24-h. On the contrary, in Star leaves MATE increase ~2-fold in all treatments at 48-h. In conclusion, Al-treatment increase Al-concentration, but down-expression ALMT and MATE genes to short-term in blueberry.

Keywords: Aluminum-activated Malate Transporter (ALMT), Multidrug and Toxic Compound Extrusion or Multi-Antimicrobial Extrusion (MATE), aluminum toxicity.

Acknowledgment: FONDECYT Regular 1201749, 1211856 and FONDECYT Postdoctorado 3220674 projects.

"NUAK1 regulates lysosome biogenesis through TFEB subcellular localization in cancer cells"

Viviana Coliboro[1], Mario Palma[1], 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.

NUAK1 is a serine/threonine kinase overexpressed in cancer and associated with poor prognosis. It supports tumorigenesis by promoting cancer cells' survival, migration, invasion, and protective stress response. However, it is unclear how NUAK1 is molecularly involved in these processes. Recently, we studied NUAK1's role in promoting cancer cell integrity by regulating lysosome biogenesis, an organelle involved in apoptosis and autophagy crosstalk, whose role in cancer has gained particular relevance in recent years. By lysotracker immunocytochemical studies in MDA-MB-231 cells, we found that HTH-01-015, a specific NUAK1 inhibitor, induces a continuous increase in lysosome fluorescence intensity over time, suggesting an increase in its biogenesis. Considering that lysosome biogenesis is controlled by the TFEB transcription factor (normally found on cancer cells' cytoplasm), we tested whether NUAK1 influences its localization. By immunocytochemistry, we found that NUAK1 inhibition induces TFEB nuclear localization. To confirm that the nuclear TFEB accumulation results in its transcriptional activation, we performed RT-qPCR to detect TFEB target genes. We found that HTH- 01-015 at 2 hours after treatment increases TFEB targets expression. Consistently, NUAK1 inhibition increased LAMP1/CTSD protein levels. Our results suggest that NUAK1 contributes to cancer cell integrity by regulating lysosomal biogenesis through TFEB cytoplasmic retention. Interestingly, a bioinformatic analysis predicts potential phosphorylation residues for NUAK1 on TFEB. Particularly, S467 coincides with a residue phosphorylated by AKT, which promotes TFEB cytoplasmic retention, suggesting that NUAK1 regulates TFEB localization similarly. However, additional studies are necessary to elucidate this mechanism.

Funding: This work was supported by the National Fund for Scientific and Technological Development (FONDECYT Regular: 1201215 and 1191172).

"Characterization of SHATTERPROOF (SHP), a transcription factor probably involved in softening of *Fragaria chiloensis* fruit"

Macarena Z. Curaqueo[1], Raúl Herrera[1], M. Alejandra Moya-León[1]

[1]Functional Genomics, Biochemistry and Plant Physiology Group, Instituto de Ciencias Biológicas, Universidad de Talca, Talca, Chile.

Fruit softening takes place during ripening, the last phase of fruit development. Softening is related to cell wall disassembly, which is due to the participation of a series of cell wall degrading enzymes that participates in a coordinated and orchestrated manner. The genes codifying for these enzymes display an increment in expression during *F. chiloensis* softening. The promotor sequences of these genes were obtained, and the analysis indicated that they contain some cis elements recognized by transcription factors (TF). Therefore, we initiate the study of the participation of TFs in softening regulation. A MADS-box type TF was identified in a transcriptome of *F. chiloensis* fruit and named SHATTERPROOF (SHP). The CDS sequence was obtained (*FchSHP*, 758 bp) and codifies for a peptide of 28.7 kDa. *FchSHP* displays an increment in expression as the fruit ripens. The CDS was cloned and expressed in *E. coli*. The purified protein was used in EMSA assays demonstrating that it could interact with DNA sequences containing cis acting elements with MADS signature CArG-box. As MADS-box TFs interact with other TFs, the interaction of FchSHP with itself and with FchSEP3, another TF previously identified, were studied. Two techniques were used to test protein-protein interaction: yeast two hybrid assay and Bimolecular fluorescence complementation (BiFC). The results indicate that FchSHP can form a heterodimer with FchSEP3, FchSEP3 can established homodimers, while FchSHP cannot interact with itself. All these information provide clues to understand the regulation of softening in *F. chiloensis* fruit.

Funding: This project was supported by ANID/FONDECYT Project (number 1210948).

"HDAC6 regulates the transcriptional activation of STAT3 pathway by altering the STAT3-PP2A association in colorectal cancer cell lines"

M. Estrella Armijo[1], Christian Zevallos[2], Alejandro Villagra[2], Matías I. Hepp[1]

[1] Laboratorio de Investigación en Ciencias Biomédicas, Departamento de Ciencias Básicas y Morfología, Facultad de Medicina, Universidad Católica de la Santísima Concepción, Concepción, Chile
[2] Lombardi Comprehensive Cancer Center, Georgetown University, Washington DC, United Stated of America

Cancer is the leading cause of death in Chile. Colorectal cancer (CRC) is a frequent type of cancer and the mechanisms associated on resistant and/or ineffectiveness of current treatments are poorly understood. HDAC6 is a histone deacetylase, mostly localized in cytoplasm. In CRC, activation of HDAC6 upregulates the gene expression of negative modulators of the immune system. However, the mechanism by which HDAC6 promoted this transcriptional change in CRC cells is not fully understood. STAT3 is a transcription factor that translocate from the cytoplasm into the nucleus when its activated. Upregulation of STAT3 is oncogenic and could lead to cancer cells evasion of the immune system. In melanoma, HDAC6 interacts with and upregulates STAT3, increasing levels of p-STAT3 (Tyr705) and its translocation to the nucleus. Furthermore, loss of HDAC6 promotes the interaction between STAT3 and PP2Ab, a regulatory subunit of PP2A phosphatase involved in dephosphorylation and inhibition of STAT3. PP2A is an important regulator of STAT3, so the effect that HDAC6 had on the STAT3-PP2A interaction may occur in CRC where HDAC6 and STAT3 pathway are upregulated. Using in silico analysis, we observed that HDAC6 had a higher affinity for STAT3 than PP2A, suggesting a competitive behavior over STAT3. Through immunoprecipitation, we show that HDAC6 interacts with STAT3 in CRC cell lines, and overexpression of HDAC6 diminished STAT3-PP2Ac (catalytic subunit) interaction. Interestingly, HDAC6 co-immunoprecipitated with PP2Ac. Here we showed that HDAC6-STAT3-PP2A axis exist in CRC, which provide insight into HDAC6 signaling mechanisms and its potential as therapeutic target in CRC.

Funding: This work was supported by ANID, through FONDECYT iniciación (11190287) to MIH. Dirección de Investigación UCSC, through convocatoria Posiciones Postdoctorales UCSC 2021 to MEA.

NUAK1 as a potential regulator of HnRNPK

Alejandro Farías[1], Viviana Coliboro[1], Sebastián Azocar[1], Mario Palma[1], Maximiliano Figueroa[2], Roxana Pincheira[1], Ariel Castro[1]

[1] Laboratorio de Transducción de señales y Cáncer, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile.
[2] Laboratorio de Biofísica Molecular, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile.

NUAK1 is a serine/threonine kinase overexpressed in multiple types of cancer and associated with poor prognosis. It supports tumorigenesis by promoting cancer cells' survival, migration, invasion, and RNA metabolism. However, it is unclear how NUAK1 is molecularly involved in these processes. We searched for new NUAK1 interactors using MudPIT analysis. We found that NUAK1 could associate with heterogeneous nuclear ribonucleoprotein (HnRNPK), which participates in mRNA regulation processes, such as stability, translation, and splicing. Analysis of public RNA-seq data of both NUAK1- and HnRNPK-knockdown cancer cells showed a strong overlapping of differential expressed genes, suggesting a functional interaction between NUAK1 and HnRNPK. Using HCT116 colorrectal cancer cells, we validated the association between NUAK1 and HnRNPK through coimmunoprecipitation assays. Confocal microscopy and image analysis showed co-distribution of NUAK1 and HnRNPK in the nucleus. On the other hand, a bioinformatic analysis predicted potential HnRNPK phosphorylation residues for NUAK1, located in the RNA binding domains of HnRNPK. In addition, a molecular docking model showed that the kinase domain of NUAK1 interacts with an RNA binding domain of HnRNPK, where one of the putative phosphorylation residues localizes. These results suggest that NUAK1 phosphorylates HnRNPK, regulating its interaction with RNA partners.

Funding: FONDECYT 1201215 and 1191172.

"Polycystin-2 and Beclin-1 (PKD2/BECN1): A protein complex in the primary cilium of POMC neurons"

Camila García-Navarrete[1,2,3], Catalina Kretschmar[2,3], Daniel Peña-Oyarzún[2,3,4,5] Eugenia Morselli[4,6,7], Valentina Parra[1,3*], Alfredo Criollo[2,3,6]

[1] Department of Biochemistry and Molecular Biology, Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile.
[2] Instituto de Investigación en Ciencias Odontológicas, Facultad de Odontología, Universidad de Chile.
[3] Advanced Center for Chronic Diseases (ACCDiS), University of Chile, Santiago, Chile.
[4] Physiology Department, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, Chile.
[5] Interdisciplinary Center for Research in Territorial Health of the Aconcagua Valley (CIIS-Te Aconcagua), School of Medicine, Faculty of Medicine, San Felipe Campus, Universidad de Valparaíso, Chile
[6] Autophagy Research Center, Santiago, Chile.
[7] Department of Basic Sciences, Faculty of Medicine and Sciences, Universidad San Sebastián, Santiago, Chile.
*Research Communication Sponsor

The primary cilium is an organelle capable of sensing extracellular stimuli that activate the signaling pathways necessary for maintaining tissue homeostasis. Recent studies have demonstrated that the primary cilium is required to activate autophagy, an intracellular mechanism that recycles proteins and organelles in specialized compartments called autophagosomes. In addition, it has been shown that both abnormal ciliary function and autophagy dysregulation in hypothalamic POMC neurons are implicated in developing metabolic diseases and obesity. Interestingly, our group has shown that PKD2/polycystin-2, a protein enriched in the primary cilium, is not only needed and sufficient for the induction of autophagy, but also interacts with Beclin-1/BECN1, a critical protein of the autophagy machinery. Thus, we hypothesize that PKD2 and BECN1 are forming a protein complex in the primary cilium in an in-vivo model of POMC neurons (N43/5 cells). Through confocal fluorescence microscopy, we analyzed the localization of PKD2 and BECN1 at the primary cilium; and by using specific ciliary markers, such as acetylated tubulin and ARL13B, we found that both PKD2 and BECN1 co-localize in cilia.

In addition, our findings revealed that PKD2 and BECN1 co-immunoprecipitated, suggesting the formation of the ciliary protein complex PKD2-BECN1. Altogether, our data suggest that the regulation of autophagy by the primary cilium may be mediated by the PKD2-BECN1 complex, which could be key to developing new treatments for metabolic diseases in which POMC neurons are implicated.

Funding: This project was funded by FONDECYT 1190743 (VP) and 1211329 (AC), ANID FONDAP 15130011 (VP, AC) and ICGEB CRP/CHL18-04 (VP).

Generation of specific Nanobodies against SARS-CoV-2 Nucleoprotein in alpacas in Chile

Angélica Bravo[1,2#], Constanza Salinas-Varas[1#] , Guillermo Valenzuela-Nieto[1], Ronald Jara[1], Yorka Cheuquemilla[1], Anne Berking[2], Romina Russo[3], María Teresa Damiani[3], Alejandro Rojas-Fernandez[1,2]

[1]Institute of Medicine and Center for Interdisciplinary studies on the nervous system, CISNe, Faculty of Medicine, Universidad Austral de Chile, Valdivia, Chile.
[2]Berking Biotechnology, Valdivia, Chile.
[3]Biochemistry and Immunity Laboratory, Medicine School, Universidad de Cuyo, Mendoza, Argentina.
#Speakers

Despite unprecedented global efforts to rapidly develop SARS-CoV-2 treatments and rapid diagnostic tools, the virus remains a worldwide concern. In order to reduce the burden placed on health systems, effective diagnosis, treatment, and prophylactic measures are urgently required to meet global demands: recombinant antibodies fulfill these requirements with marked clinical potential. SARS-CoV-2 Spike, and Nucleoprotein (NP) are the principal targets of the host's humoral response. Unlike Spike, the NP is a more stable and reliable candidate for diagnostic due to its low mutation rate. This study shows the generation of alpaca Nanobodies (Nb) specific for SARS-CoV-2 NP. Here, we characterize two nanobodies isolated from a VHH library in *E. coli* surface display, which allows single-step selection of the Nb using a simple density gradient centrifugation of the bacterial library. The nanobodies NP21 and NP147 bind selectively to the SARS-CoV-2 NP when tested in immunoassays and can be used as a low cost biotechnology tool for direct diagnostic of SARS-CoV-2.

This work was supported by Berking Biotechnology and the Universidad Austral de Chile

"Transcriptional networks of bZIP25 regulates endocytic trafficking in *Arabidopsis thaliana*"

Carolina Galleguillos [1], Claudio Osorio-Navarro[1], Damián Cifuentes1, Lorena Pizarro[2] & Lorena Norambuena[1]

[1] Centro de Biología Molecular de Plantas, Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile
[2] Instituto de Ciencias Agroalimentarias, Animales y Ambientales. Universidad de O´Higgins, San Fernando, Chile

The *bZIP* genes encode proteins with a conserved basic DNA-binding motif and a leucine zipper domain required for dimerization. The *Arabidopsis thaliana AtbZIP* family includes 75 members, many of whom have an elusive function. The characterized bZIP members are involved in plant homeostasis and signaling, cell differentiation and growth, and biotic and abiotic stress responses. These processes are supported and regulated in different aspects by protein trafficking dependent on the endomembrane system. We have been working with several AtbZIP members based on genetic evidence obtained in our lab that relate their role with endomembrane trafficking modulation. The loss of function mutant displayed an accelerated endocytosis phenotype, which was rescued by the *AtbZIP25* overexpression, indicating that *AtbZIP25* negatively regulates endoctitic pathway. With the aim of understanding the gene regulatory network of *AtbZIP25*, the transcription profile of the mutant was analyzed and compared to the wild-type line. The loss of function *AtbZIP25* induced changes in 154 genes related to endomembrane trafficking; 67 out of them were downregulated in the mutant. When the endocytic trafficking is chemically perturbed, there is a transcriptional induction on processes related to endocytosis, endosomal transport, and vesicle trafficking on the wild-type line. However, this is missing on the loss of function of *AtbZIP25*. Our results shed light on the role of the *AtbZIP25* in the transcriptional regulation of genes involved on endomembrane trafficking in plant cells.

Funding: This work was supported by Fondecyt 1211311

"The relationship of RNA m6A machinery with hypertrophy in neonatal ventricular rat myocytes induced by norepinephrine"

Sebastián Urquiza-Zurich [1], Paulo Amaral [2], Sergio Lavandero [1], Vinicius Maracaja-Coutinho [1]

[1] Advanced Center for Chronic Diseases - ACCDiS, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.
[2] Insper Instituto de Ensino e Pesquisa, São Paulo, Brasil.

Cardiac hypertrophy (CH) is a compensatory response to pressure overload, myocardial damage and is characterized by the activation of signaling cascades that promote the expression of specific genes and increase the size of the cardiomyocyte. The molecular mechanisms that occur in its genesis and development are still completely unknown. Epitranscriptomics at the level of transcripts is an emerging field in the study of the pathogenesis of CH and the N6-methyladenosine (m6A) modification is one of the most abundant and predominant in eukaryotic transcripts. However, the role of m6A methylation in CH context is still unknown. There is evidence of several in vitro and in vivo hypertrophic stimuli, but not with norepinephrine (NE) stimulus and its relationship with RNA m6A machinery. Therefore, we proposed that RNA m6A machinery is dysregulated in hypertrophy processes, specifically in a NE-induced context. In this work, we stimulated neonatal ventricular rat myocytes (NVRMs) with 10 μM of NE in different times (0, 6, 18, 24 and 48 hours) to evaluate the expression and protein content of the RNA m6A machinery (writers, erasers, readers), as well as hypertrophic markers (ANP, BNP and β-MHC) by RT-qPCR and Western Blot, respectively, in a condition with and without hypertrophic stimulus. As a result, we obtained that there is an upregulation of the eraser proteins (FTO and ALKBH5) at the 24 and 48 hours when we stimulated with NE. In this way, understanding the epitranscriptome characteristics at the cardiac level, specifically in a cell-specific context, is relevant for the development of more effective and direct therapies.

Funding: This work was supported by grants from the Agencia Nacional de Investigación y Desarrollo (ANID), Chile: FONDECYT 1211731 (VM) and Proyecto FONDAP 15130011 (SL, VM).

"Assessing adaptive advantages and evolutive implications behind acquisition and loss of DNA regions by horizontal gene transfer events in yeast"

Andrés Romero [1,2], Pablo Villarreal [2,3], Francisco Cubillos [2,3], Francisco Salinas [1,2]

[1] Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile.
[2] ANID-Millennium Science Initiative-Millennium Institute for Integrative Biology (iBio), Santiago, Chile.
[3] Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile.

In eukaryotes, horizontal gene transfer (HGT) is thought to occur at low frequency due to cellular barriers to incorporating foreign genetic material and possible transcriptional and translational incompatibilities. In *Saccharomyces cerevisiae*, almost 185 ORFs seem to be acquired by HGT but there is scarce functional evidence demonstrating their adaptive advantages and biological relevance. Here, we evaluated a region absent in the reference genome described as region B (~17 Kb coding 5 ORF) acquired from *Zygosaccharomyces baili*. We used the CRISPR-Cas technology to obtain an artificial ancestor of the wild strain AHG (region BΔ strain) that simulates a previous stage of the HGT event to compare its phenotype respect to the wild-type. As result, microcultivation experiments suggest a role of region B in AHG strain adaptation to different carbon source utilization, nitrogen concentrations, and osmotic stress. Besides, we generated transcriptional reporters using the 5 promoters of the region coupled with the luciferase gene to assess the contribution of each gene to yeast fitness. The results confirmed an active and differential process of transcription of the region B in the AHG strain and a remarkable absence of activity for the BY4741 strain in synthetic complete (SC) medium. Altogether, we confirm that region B confers adaptive advantages to the AHG strain respect to its artificial ancestor, suggesting the necessity of selective pressure for the integration and fine-tuning of the molecular interactions of a foreign region with its new host.

This work was supported by ANID-FONDECYT 1210955.

Production and characterization of extremophile bacterial pigments to produce Dye Sensitized Solar Cells (DSSC)

Jose Martínez-Oyanedel [1] ,I sabel Alarcón [1], Catalina Espinoza [1], Helia Bello [2], Miguel Martínez [2], Paulraj Manidurai [3]

[1] Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción
[2] Departamento de Microbiología, Facultad de Ciencias Biológicas Universidad de Concepción
[3] Departamento de Física, facultad de Ciencias Físicas y Matemáticas, Universidad de Concepción

The energy obtained from the sun can be capture in solar cells. The conventional ones are made of silicon crystalline or gallium arsenide, these, although highly efficient, they have a high cost of production and the reactions that are used to produce silicon achieve conduct electricity generate more contamination, this is why the solar cells dye-sensitized (DSSC) are a sustainable and low-cost way to obtain renewable energy.
Organic pigments, ruthenium or platinum complex has been used as Photosensitizers, because are more expensive or difficult to obtain, natural pigments have been used to prepare DSSC. Pigments can be extracted from fruits, leaves, flowers, bacteria, algae, among others sources, of these the most common of used for DSSCs are flavonoids, anthocyanin and beta carotene.
Pigment produced from bacteria has been a viable alternative, due to its high availability, low cost of obtaining and easy extraction procedures.
In this project we produce several pigments cultivating Antarctic bacteria. All pigments were obtained from the bacterial cells using methanol. UV-Visible and fluorescence spectrum characterization of each pigments was performed.
The pigments were used to sensitized, TiO_2 anodes and cells were assemblies using the redox pair $I^-/I3^-$ as electrolytic solution. The cells were characterized in the efficiency to transform the radiative energy in electricity.
Only some of the pigments were able to produce electricity in el cells assemblies.

Funding: VRID 2021000331MUL

"The glutamate receptor-like channels (GLRs) at Antarctic moss, *Sanionia uncinata*, and its contribution to ROS balance during abiotic stress"

Alex San Martín-Davison[1], Oscar Arrey-Salas[1], Mónica Yánez[1], Erwan Michard[1]

[1] Laboratory of Plant Cell Environment Sensing and Signaling (PCESS Lab), Instituto de Ciencias Biológicas, Universidad de Talca, Talca 3460000, Chile

A variety of environmental stresses, such as temperature, dehydration, salinity, and UV radiation, have a negative impact on plant life cycle and productivity. At the cellular level, one detrimental aspect common to all those abiotic stressors is the generation of toxic reactive oxygen species (ROS) by dysfunctional chloroplasts and mitochondria. In the signaling pathway leading to plant adaptation to abiotic stresses, a group of ion channels, so called glutamate receptor (GLR), have been identified that play an important role, through calcium and electric signaling. Interestingly, GLRs were recently shown to be regulated by ROS, but the physiological meaning of this regulation is still unknown. To investigate a role of GLR in abiotic stress sensing through ROS regulation, we used the Antarctic moss *Sanionia uncinata*, a remarkable specie characterized by a hyper tolerance to different types of environmental stresses. We evaluated the gene expression profile of SuGLR1 during an assay where plants were subjected to different temperatures (5, 15, 21 and 28°C). The *SuGLR1* mRNA accumulation were contrasted with the level of transcripts of the genes coding for enzymes involved in ROS scavenging and buffering (*APX, SOD, POX and CAT*) as well as with the analysis of the corresponding enzyme activity obtained from the extracts derived from each treatment. We will discuss the results in term of GLR involvement in plant abiotic stress response and put in perspective the case of the Antartic moss adapted to survive in extreme conditions compared to canonical mosses and plants.

Funding: This work was supported by Fondecyt Regular1210920 and post-doctoral fellowship from University of Talca.

"Specific proteolysis mediated by a p97-directed proteolysis-targeting chimera (p97-PROTAC)"

Constanza Salinas-Rebolledo[1], Javier Blesa[2], Guillermo Valenzuela-Nieto[1], David Schwefel[3], Natalia López González del Rey[2], Maxs Méndez-Ruette[4], Janine Burkhalter[1], Luis Federico Bátiz[5 & 6], Yorka Cheuquemilla[1], Ronald Jara[1], José A. Obeso[2], Pedro Chana-Cuevas[7], Gopal P. Sapkota[8], Alejandro Rojas-Fernandez[1]

[1] Institute of Medicine, Faculty of Medicine, Universidad Austral de Chile, Valdivia, Chile.
[2] HM CINAC (Centro Integral de Neurociencias Abarca Campal), Hospital Universitario HM Puerta del Sur, HM Hospitales, Madrid, Spain
[3] Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany
[4] Programa de Doctorado en Biomedicina, Facultad de Medicina, Universidad de los Andes
[5]Neuroscience Program, Centro de Investigación e Innovación Biomédica (CiiB) & School of Medicine, Facultad de Medicina, Universidad de los Andes, Santiago, Chile.
[6] IMPACT, Center of Interventional Medicine for Precision and Advanced Cellular Therapy, Santiago, Chile.
[7] CETRAM & Faculty of Medical Science Universidad de Santiago de Chile, Chile.
[8] Medical Research Council Protein Phosphorylation and Ubiquitylation Unit, School of Life Sciences, University of Dundee, Sir James Black Centre, Dundee, UK

Targeted degradation of endogenous proteins using PROTAC (*"proteolysis targeting chimera"*) technology has emerged as a new therapeutic tool to address diseases driven by aberrant protein expression and/or aggregation. Through E3 ligase enzymes, these systems are capable of ubiquitinating specific proteins to be subsequently degraded through the ubiquitin-proteasome system (UPS). The p97 protein is a member of the AAA^+ family of ATPases. It is a mechanoenzyme that uses energy from ATP hydrolysis to actively promote protein unfolding and segregation. The unfolded products of p97 are presented to the 26S proteasome for degradation. p97 substrate recognition is mediated by cofactors, which interact with substrates directly or indirectly through ubiquitin-modifications, resulting in substrate funneling into the central pore of the p97 hexamer and unfolding. We have engineered synthetic adaptors to target specific substrates to p97, using the extraordinary intracellular binding capabilities of camelid nanobodies fused to the UBX domain of the p97 cofactor FAF1. These adaptors were tested in cell lines, where a significant decrease in protein levels was observed. In such a way, we created a p97-directed proteolysis-targeting chimera (p97-PROTAC), representing a unique E3 ubiquitin ligase-independent strategy to promote specific proteolysis.

Funding
This work was funded by FONDECYT REGULAR 1200427; FONIS EU-LAC T010047; FONDEQUIP EQM180037; the regional Council of the “Los Rios region” project FICR16-19, FICR19-20 and FICR21-01

"Influence of the LZ domain on the structural and functional properties in the human transcription factor FoxP1"

Isabel Asela[1], Perla Cruz[1] ,Arturo Lamilla[1], Yerko Castillo[1] ,Jorge Babul[1], Exequiel Medina[1]

[1]Laboratory of Biochemistry and Molecular Biology, Department of Biology, Faculty of Sciences, University of Chile, Santiago, Chile.

The human transcription factor FoxP1 is structurally characterized by being a multidomain protein that contains a canonical intrinsically disordered region known as leucine zipper (LZ) and a DNA-binding domain (FKH) that dimerize in absence of DNA. In this work we seek to understand the interdomain LZ-FKH communication to determine its structural and biophysical implications in FoxP function.
We used the bidomain LZ-FKH specifically labelled either in LZ or FKH domain with a fluorescent dye to evaluate local changes in dissociation and ligand binding assays. Equilibrium dissociation studies showed that the Kd value in bidomain LZ-FKH is 50 times lower than the isolated FKH domain, suggesting that the dimeric state is strongly favored when the LZ domain is present. On the other hand, equilibrium unfolding studies indicated that the stability of the FKH domain is not affected by the presence of the LZ compared with the isolate FKH, while the stability of the LZ domain in the bidomain LZ-FKH is 5 kcal/mol lower than the FKH, suggesting that this domain effectively is not disordered as observed in other protein models. Finally, DNA binding studies indicated that the presence of the LZ domain does not have impact in the FKH-DNA affinity, suggesting that, different to other FoxP members, the LZ domain could not be involved in the association between FKH and DNA. Altogether, the presence of the LZ has heterogeneous consequences in FoxP1, mainly increasing the dimeric state, affecting therefore the regulatory aspects regarding the repressive rol of FoxP transcription factors.

Funding: This work was supported by Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT) grant Nº11200729

"Biophysical characterization of the acetylation mimetic mutants K359Q and K372Q in the leucine zipper domain of the human transcription factor FoxP1"

Dayanna Nuñez[1] ,Yerko Castillo[1], Jorge Babul[1], Exequiel Medina[1]

[1]Laboratorio de Bioquímica y Biología molecular, Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile.

The human transcription factor FoxP1 is a member of the Fox family of transcription factors, that plays a fundamental role in the regulation of gene transcription in processes like embryogenesis and immunity. Structurally, FoxP proteins are characterized by containing a highly conserved DNA-binding (forkhead, FKH) and a leucine zipper (LZ) domains, actively modulating the regulatory role as transcription factors. Additionally, some acetylation modifications have been described in FoxP proteins affecting their function. Lysine residues 359 and 372 in the LZ domain have been postulated as the main target for these post-translational modifications. However, there is a scarce structural and functional information regarding the presence of these acetylation in the LZ domain in the properties of FoxP proteins.

In this research, we used the bidomain LZ-FKH of FoxP1 to generate acetylation-mimetic mutants (lysine-to-glutamine) in residues 359 and 372, characterizing their biophysical properties such as dissociation, stability and ligand binding. Our equilibrium dissociation assays indicated that the mutant K359Q increased its Kd ~100-fold, whereas the mutant K372Q behaves like an obligated dimer at concentration even lower than 10 nM. Equilibrium unfolding experiments performed showed that neither the stability of the LZ nor the FKH domains are affected by K372Q substitution, although the fold of the LZ is lost in the mutant K359Q. Finally, DNA binding experiments indicated that the substitution K372Q increased the affinity for DNA, whereas the substitution K359Q does not have an apparent impact. These results suggest that each acetylated lysine has an specific effect in the LZ-FKH of FoxP1

Funding: This work was supported by Fondo Nacional Científico y Tecnológico (FONDECYT) grant N°11200729

Characterization of the acid-sensitivity of the Andes orthohantavirus Gn/Gc glycoproteins in the Golgi apparatus and endoplasmic reticulum

Fabián Figueroa[1,2], Nicole Tischler[1,3]

[1] Laboratorio Virología Molecular, Centro Ciencia & Vida, Fundación Ciencia & Vida, Santiago, Chile
[2] Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago, Chile
[3] Departamento de Ciencias Básicas, Facultad de Medicina y Ciencia, Universidad San Sebastián, Santiago, Chile

The Andes orthohantavirus Gc glycoprotein forms part of a hetero-octameric Gn/Gc spike complex and is responsible to induce the virus-cell membrane fusion during viral entry into host cells. A key factor for this process is the exposure of Gc to mildly acidic pH, that triggers a irreversible conformational change of the Gn/Gc spike into a Gc homotrimer characterized by its high stability and resistance to digestion by enzymes like trypsin. Yet, the assembly and priming mechanism which leads to the acid-sensitive hetero-octameric Gn/Gc complex once the virus is outside the cell, while keeping a resistance to mildly acidic in the secretory pathway pH to avoid premature activation is completely unknown. To asses this question, we aim to separate the Gn/Gc glycoproteins in the endoplasmic reticulum (ER) and Golgi apparatus and characterize their resistance to acid activation. Using iodixanol gradients and staining of well established marker proteins, we identified fractions which include the presence of ER and Golgi markers and their colocalization with the Gn/Gc proteins. Exposing these fractions to a pH of 5,5 will allow us their characterization in terms of Gc homotrimerization and resistance to trypsin. The results will be key to better understand the assembly and maturation process of hantaviruses.

Funding: ANID (Chile) grants FONDECYT 1221811 and Centro Ciencia & Vida BASAL FB210008 (Excellence Center).

"Rebirth after the pandemic situation: structural biology applied to new and old problems"

Maximiliano Figueroa, Catalina González, Florencia Quiroz, Dory Toledo, José Martínez-Oyanedel

Laboratorio de Biofísica Molecular, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción

The (still) current pandemic situation due to the Sars-Cov-2 virus and COVID has affected the whole society. At academic level the pandemic produced a break in the scientific production, due to the impossibility to attend to the laboratories, complicated schedules, or break in the supplier's chain. Our laboratory was not allowed to engage new students during 2020 and 2021, meaning that after graduation of the current students of those years the lab was empty. 2022 arrived with new air, and the possibility to re start all the academic activities. New undergraduate students have been engaged in our laboratory and with them new projects started. Histamine intolerance is a condition related to allergies or food intolerance, where the person has a reduced histamine degradation in the intestine due to impaired diamine oxidase (DAO) activity. The people suffering from this condition have several symptoms such as headaches, gastrointestinal symptoms, acne, and others. The consumption of this enzyme may reduce the effect of histamine in the intestine. Currently, DAO from pig kidney is offered as an alternative, but extracted in a native way. We are developing a recombinant production of this enzyme. Regarding artificial proteins, we are working in the design of peptides with therapeutic potential over infection by human papilloma virus (HPV). This peptide will allow to disrupt the interaction of a viral protein with p53 in the cell, avoiding the degradation of p53. In silico experiments demonstrate that our design is going in the right direction. Our last project is the design of an artificial protein with exceptional nutritional capacities. This artificial protein is designed to be degraded up to free essential amino acids in order to allow a better absorption. This protein could be used as a food supplement, special ingredient in survival kits, or even as super food for the military or space travel.

Funding: no funding

"Structural and functional characterization of ancestral arsenic-binding proteins"

Consuelo Badilla[1] , Pablo Cea [1], Victor Castro[1], Victoria Guixé [1]

[1] Laboratorio de Bioquímica y Biología molecular, Biología, Ciencias, Universidad de Chile, Santiago, Chile.

In bacteria, a sub-family of periplasmic-binding proteins (PBP's) that bind arsenic-oxyanions (As^{+3} – As^{+5}) have been recently described, comprised by AioX, and its orthologues ArxX and ArrX, which show different oxyanion specificities. In this work we addressed the evolutionary history of ligand specificity through the reconstruction and characterization of ancestral proteins from this family. Specifically, we reconstruct the last common ancestor of ArrX (AncROX), ArxX (AncOX), and AioX (AncO) to identify the structural determinants responsible for the generation of ion specificity. The phylogenetic tree for this family was generated by collection of a total of 1000 sequences for AioX, ArxX, and ArrX using maximum likelihood and the LG+I+G+F evolutive model. Ancestral proteins were purified from recombinant expression in *E. coli* str BL21. Crystal structure of ancestral proteins were determined and phasing was solved by molecular replacement using AioX and AlphaFold modelling as template. The structures show a similar topology to extant PBP's with two globular regions connected by a hinge, forming a groove between them where bonded arsenite is completely buried. Ligand binding specificity was determined by intrinsic fluorescence and ITC measurements. The results show that all ancestral proteins of the sub-family of periplasmic-binding proteins (PBP's) are capable of binding arsenic-oxyanions. In particular, the most ancestral protein (AncROX) can bind either As^{+3} or As^{+5}, but the affinity for As^{+5} being higher. In contrast, the most recent ancestor (AncO) has more affinity by As^{3+} than As^{+5}, showing that As^{3+} specifity is the evolutionary novelty in this sub-family of periplasmic-binding proteins in bacteria.

Funding: This work was supported by Fondecyt Postdoctorado (3200737), Fondecyt (1191321), ANID.

"Mechanical pulling of artificial deeply knotted proteins"

Rodrigo Rivera[1], Andrés Bustamante[1], Oscar Aránguiz[1], Mauricio Baez[1]

[1] Laboratorio de Bioquímica, Departamento de Bioquímica y Biología Molecular, Universidad de Chile, Santiago, Chile.

Knotted topologies can remain in the unfolded states of knotted proteins even in strong denaturant conditions. Nonetheless, the dynamics of knots in unfolded states is poorly understood. In silico studies has proposed that deeper knots can increase the energy barrier to unknot the polypeptide chain upon unfolding. Nevertheless, there is a lack of experimental data in this regard. To address this problem, we pull an artificial knotted protein containing a deep trefoil knot (2out-knot) from different pulling points in order to untie its polypeptide chain using optical tweezers. Force-ramp experiments showed trajectories with different number of transitions depending on the pulling points used to stretch the protein associated to unfolding and refolding events. These events were grouped in clusters indicating different unfolded sates. Three out five pulling points showed a contour length variation (ΔLc) in agreement with the expected value for the fully unfolded-unknotted protein. These data suggest that the protein can be fully unfolded-untied, or the protein remains trapped in a knotted intermediate. Despite of these, the kinetic analysis showed that these events are not accompanied with a rise in the folding barrier, which is in direct conflict with previous in silico studies and additional evidence. The results suggest that the ΔLc on its own is not a suitable parameter to follow the topologic variations on the polypeptide chain, since this approach not consider the intrinsic properties of the polymer chain like the stretching modulus. Hence, it's not clear if the protein can be unknotted during the mechanical perturbation.

Funding: FONDECYT 1191153.

"Characterization of the spikes from different bunyaviruses"

Esteban Rodríguez[1,2] , Gianina Arata[1], Eduardo A. Bignon[1,2], Nicole D. Tischler[1,2]

[1]Laboratorio de Virología Molecular, Centro Ciencia & Vida, Fundación Ciencia & Vida, Santiago, Chile.
[2]Departamento de Ciencias Básicas, Facultad de Medicina y Ciencia, Universidad San Sebastián, Santiago, Chile.

The Bunyavirales order include a large group of human pathogenic viruses, such as hantaviruses, phleboviruses and nairoviruses. They include a lipid envelope projecting Gn/Gc glycoprotein spike of different multimerization order with Gc having a class II fusion protein fold. The viral spikes induce virus cell entry by receptor interaction and subsequent virus-cell membrane fusion. Also, because the spikes are surface exposed, they are the main targets of neutralizing antibody responses, and hence represent attractive antigens to induce protective immunity. Some viruses, display a "breathing" behavior, where the spikes undergo a dynamic fluctuation leading to exposure or mask neutralizing epitopes. In this work, we aim to study whether different bunyavirus spikes may show dynamic fluctuations, as we have previously shown for hantaviruses. To test this, we are preparing virus-like particles and corroborated their reactivity with monoclonal antibodies. The exposure of internal fusion loops of the Gc fusion protein is assessed with liposome co-flotation assays in dependence of different temperatures. The results that we have obtained so far from Rift valley fever virus-like particles show, that at low temperature, a membrane-interacting and non-interacting fraction exist, which can be shifted towards full liposome interaction at high temperature (50 ºC). Yet, it remains to be determined if this observation can be related to fusion loop exposure and whether both forms may be at a dynamic equilibrium.

Funding: This work was supported by the FONDECYT (number 1221811) of the Agencia Nacional de Investigación y Desarrollo (ANID, Chile) and Centro Ciencia & Vida BASAL (number FB210008) (Excellence Center).

"Molecular redesign of an optogenetic switch in yeast"

Diego Ruiz[1,2], David Figueroa[1,2], Camila Baeza[1,2], Andrés Romero[1,2], Francisco Salinas[1,2].

[1]Laboratorio de Genómica Funcional, Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile.
[2]ANID–Millennium Science Initiative–Millennium Institute for Integrative Biology (iBIO), Santiago, Chile.

Saccharomyces cerevisiae is an organism widely used for the study of fundamental genetics and engineering of new fuels, chemicals, and pharmaceuticals. However, expressing heterologous proteins requires high levels of gene expression. In this sense, light has shown to be an excellent inducer of gene expression with multiple advantages compared to chemical inducers, such as its low cost, low toxicity to cells, spatiotemporal resolution, and reversibility. On the other hand, optogenetic switches are molecular systems based on photosensitive proteins or photoreceptors. One of these tools, developed from fungal blue light photoreceptors, is the FUNgal Light-Oxygen and Voltage (FUNgal Light-Oxygen and Voltage) optogenetic switch. This optogenetic system enables light-controlled gene expression of any target gene in yeast. In this study, the FUN-LOV optogenetic switch was redesigned, generating a new variant (FUN-LOVSP) that includes the PPGK1 and PTDH3 promoters, and it was cloned in a single plasmid. Furthermore, we included antibiotic resistances (NatMx and HphMx) in the FUN-LOVSP variant, generating two additional variants (FUN-LOVSP-Nat and FUN-LOVSP-Hph). Each FUN-LOV variant was evaluated using the luciferase reporter gene under different illumination conditions. The new FUN-LOV variants showed higher levels of light-dependent luciferase expression compared to the already published systems (FUN-LOV and FUN-LOVLS). Altogether, the molecular redesign of the FUN-LOV switch generated variants with potential applications in wild and industrial yeast strains.

This work was supported by ANID-FONDECYT 1210955.

"Developing a light-regulated genetic circuit for transcriptional activation in yeast"

David Figueroa[1,2], Javiera Barría[1,2], Diego Ruiz[1,2], Claudia Inzunza[1,2], Camila Baeza[1,2], Andrés Romero[1,2], Vicente Rojas[2,3], Luis F. Larrondo[2,3], Francisco Salinas[1,2]

[1]Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile.
[2]ANID – Millennium Science Initiative – Millennium Institute for Integrative Biology (iBIO), Santiago, Chile.
[3]Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile.
Email: david.figueroa@alumnos.uach.cl
Research Communication Sponsor: Francisco Salinas

Yeast is a remarkable model organism for cellular and molecular biology studies and a biological platform for industrial and biotechnological applications such as alcoholic fermentation. The importance of the glucose fermentation pathway has prompted important efforts to redirect its metabolic flux and change the products yield. This challenge can be addressed by combining different synthetic biology tools such as optogenetics and CRISPR-Cas technologies. For instance, the FUN-LOV (FUNgal Light Oxygen and Voltage) optogenetic switch activates gene expression upon blue-light stimulation in a reversible fashion. Furthermore, CRISPR-Cas9 technology can be adapted for transcriptional activation (CRISPRa) of multiple target genes by fusing the dCas9 protein with an activation domain such as p65. In this work, we combined the reversible gene expression control of FUN-LOV with the multiplex activation of the CRISPRa system, generating a synthetic genetic circuit in yeast. We used a FUN-LOV variant (FUN-LOV$^{SP\text{-}Nat}$), integrated in the yeast genome, to control the expression dCas9-p65 protein upon light stimulation. Then, by expressing different guide RNAs (gRNAs), the dCas9-p65 protein was directed to different promoter regions (*GAL1, ADH5*, and *GPD2*), activating gene expression of a luciferase reporter. In conclusion, the synthetic circuit developed in this work has potential application in the transcriptional control of metabolic genes, changing the products balance of the glucose fermentation pathway.

This work was supported by ANID-FONDECYT 1210955 and ANID-PhD 21200745 scholarship.

"A microbial signature is specifically related to solid tumors in Chilean population"

Troy Ejsmentewicz[1], Roxana González-Stegmaier[1], Roddy Jorquera[1], Rodrigo Lagos[2] María Cristina García[3], Celia Podesta[3], Carlos Barrientos[3], Franz Villarroel-Espíndola[1]

[1] Gastrointestinal Immuno-oncology group, Translational Medicine Research Laboratory. Instituto Oncológico Fundación Arturo López Pérez (FALP), Santiago, Chile.
[2] Clinical Studies and Epidemiology Unit. Instituto Oncológico Fundación Arturo López Pérez (FALP), Santiago, Chile.
[3] Endoscopy Unit. Instituto Oncológico Fundación Arturo López Pérez (FALP), Santiago, Chile.

The human microbiome is a community of microorganisms on a particular part of the body, such as the skin or gastrointestinal tract, which coexists in equilibrium. But if there is a disturbance in that balance, dysbiosis occurs, stopping these normal interactions. The association of microbiome and their products with diseases including cancer is becoming more significant and showed that microbiome is an emerging target in tumor onset, progression, prevention, and even diagnosis. To understand the role of gut microbiome composition in Chilean oncological patients, we designed a single center and prospective clinical study based on metagenomic 16S gene sequencing. The interim analysis included 15 participants, enrolled during 2020 and 2021, segregated as benign breast disease (5), lung (1), prostate (2), uterus (1), skin (1) and testis (1), and colon (4). All groups were compared against non-oncological cases (10). The results showed no significant differences in alpha and beta diversity. As expected, the colon cancer group was characterized by a significant dysbiosis at the phylum level ($p<0.05$). High-dimensional biomarker discovery was performed by linear discriminant analysis effect size (LEfSe) method. Four categories were compared against the control group, benign breast, colon cancer and any other solid tumor combined. The phylogenetic trees showed a unique microbial signature for each group, based on their relative abundance; a heatmap representation individualized at least 2 specific species as potential biomarkers. We have discovered a specific microbiome signature for some determined tumor, which could be further investigated as a relevant diagnostic and therapeutic opportunity.

Funding: This work was supported by The National Fund for Scientific and Technological Development, FONDECYT (N° 1221415 to FVE) and Internal grant from Fundación Arturo López Pérez (FALP-LMT-2020, -2021 to FVE, and -2022 to RGS).

"Mitochondrial Ca^{2+} overload in the neurodegeneration associated with early-onset familial Alzheimer's disease"

Marioly Müller[1], Riley Payne[2], J. César Cárdenas[3] and J. Kevin Foskett[2,4].

[1]Department of Medical Technology, Faculty of Medicine, University of Chile, [3]Center for Integrative Biology, Universidad Mayor, Chile and [2]Department of Physiology & [4]Department of Cell and Developmental Biology, University of Pennsylvania, Philadelphia, USA. E-mail address: marioly@uchile.cl

Familial Alzheimer's disease (FAD) is characterized by mutations in presenilin-1 (PS1) gene affecting intracellular Ca^{2+} homeostasis. An imbalance between mitochondrial Ca^{2+} uptake and removal leads to the opening of the permeability transition pore, causing the loss of mitochondrial membrane potential ($\Delta\Psi m$), uncoupling of the respiratory chain, and a drop in ATP. Accumulation of mitochondrial free Ca^{2+} causes a toxic mitochondrial Ca^{2+} overload, inducing an apoptotic process contributing to the disease pathogenesis.
Purpose: We investigated the role of the FAD-mutant PS1-M146L disruptions of intracellular Ca^{2+} homeostasis on mitochondrial function using in vitro cell models.
Methods: We used SH-SY5Y cells transfected with mutant PS1-M146L and fibroblast from FAD, sporadic AD (SAD), and control patients. We performed simultaneous measurements of $[Ca^{2+}]m$ uptake and $\Delta\Psi m$ in permeabilized cells to measure MCU-mediated Ca^{2+} uptake. Live-cell imaging experiments were performed to estimate qualitatively and quantitatively $[Ca^{2+}]m$, using Ca^{2+} indicators.
Results: Mutant PS1 cells were subjected to elevated mitochondrial Ca^{2+} levels, but no significant differences in mitochondrial Ca^{2+} uptake through MCU or $\Delta\Psi m$ were found. Basal levels of $[Ca^{2+}]c$ and $[Ca^{2+}]m$ were significantly higher in mutant PS1 SH-SY5Y cells and fibroblasts from FAD and SAD patients. Excess Ca^{2+} levels in PS1-M146L cells triggered a sensitivity to successive Ca^{2+} challenges, causing a loss of $\Delta\Psi m$. We found a decreased expression of NCLX in mutant PS1 cells, suggesting an impaired mitochondrial Ca^{2+} efflux.
Conclusions: These features may explain increased vulnerability and eventual death due to progressive mitochondrial dysfunction, promoting a pathological cycle essential to the disease progression of FAD.

Funding: This work was supported by the University of Chile and the University of Pennsylvania

"Pepsin as an enzyme involved in the degradation of synthetic and ALS astrocytes-derived inorganic polyphosphate"

Armando Amaro, Cristian Arredondo, Polett Garces, Martin Montecino*, Brigitte van Zundert*.

Institute of Biomedical Sciences, Faculty of Medicine, Universidad Andrés Bello, Santiago, Chile. *Corresponding authors

Amyotrophic lateral sclerosis (ALS) is a devasting neurodegenerative disease caused by the selective loss of motoneurons (MNs). In our recent study (Arredondo et al., Neuron 2022), we demonstrated that mouse and human (iPSC-derived) astrocytes with diverse ALS/FTD-linked mutations (SOD1, TARDBP, and C9ORF72) display elevated levels of inorganic polyphosphate (polyP). PolyP levels are also increased in astrocyte-conditioned media (ACM) from ALS astrocytes. Using the specific yeast polyphosphatase (polyPase) PPX1, we furthermore established excessive astrocyte-derived polyP as a critical factor in MN degeneration and a potential therapeutic target for ALS. Here, we performed biochemical assays along with MN survival assays to characterized in more depth the ACM derived from mutant SOD1 ALS astrocytes (mutSOD1-ACM). Using size-fractionation and heat denaturation, our result indicates that polyP in mutSOD1-ACM is thermolabile with a molecular mass between 4-30 kDa, corresponding to a polyP chain length between 40 and 360 Pi units. Additionally, we found that MN death was prevent when mutSOD1-ACM was treated with pepsin. While these results suggest that toxic factor is of proteinaceous nature, unexpectedly, we found in additional studies that this protease cleaves polyP. Thus, assays with synthetic long-chain polyP (130 Pi) and urea-PAGE revealed that treatment with 2 commercial pepsins, but not pH2 treatment alone, led to a loss of the polyP signal with the remaining polyP staining signal smaller in size. Additional experiments were performed to determine whether pepsin acts as (similar as shown for PPX1) as an exo-polyPase or as an endo-polyPase (as shown for Nudt3).

Funding: FONDECYT 1211026 (MM) FONDECYT 1221745 (BvZ)

"*In vitro* study of boldine derivatives in viability of colorectal cancer cells"

Karla Villalobos-Nova[1], Cristián Suárez-Rozas[2], Edwin Pérez[3], Bruce K. Cassels[4] Julio C. Tapia[1]

[1] Programa de Biología Celular y Molecular, ICBM, Facultad de Medicina, Universidad de Chile, Santiago, Chile
[2] Centro de Química Médica, Facultad de Medicina, Clínica Alemana, Universidad del Desarrollo, Santiago, Chile
[3] Department of Organic Chemistry, Faculty of Chemistry and of Pharmacy, Pontificia Universidad Católica de Chile, Santiago, Chile
[4] Department of Chemistry, Faculty of Sciences, University of Chile, Santiago, Chile

Colorectal cancer (CRC) is the world's third most common type of cancer, with an incidence estimated to increase by 56% at 2040. Traditional treatment in non-advanced stages involves chemotherapy using 5-fluouracil, which is moderately effective, concomitant with side effects associated with its high toxicity. Therefore, new drugs are needed to improve CRC treatment, among which naturally derived compounds such as boldine stand out. This alkaloid, known for its antioxidant and anti-inflammatory properties, is extracted from the Chilean tree *Peumus boldus*. Possible anticancer properties have been reported in glioma, breast cancer, prostate cancer, and other cell types, but pharmacokinetic studies show that it has low bioavailability, so new strategies are required for its potential clinical use. In our laboratory, we decided to study the possible effects of boldine and 29 more lipophilic chemical derivatives against CRC cells. Cell viability was analyzed in the DLD-1 cell line, and the type of death triggered was studied by flow cytometry and a commercial cytotoxicity assay. We found that CRC cell viability differentially decreased for each compound, some mainly producing necrosis, others apoptosis, and some displaying both kinds of cell death. Future work will study the specific type of apoptosis produced and compare the effect of the most interesting compounds on non-tumor cells. Finally, we hope to relate these findings to the inhibition of the Ser/Thr protein kinase CK2 that regulates cell viability and is increased in several types of cancer, including CRC, positioning it as a possible anti-cancer target.

Funding: This work was supported by the ANID-Fondecyt grants 3220509 (CSR), 1211619 (EP), and 1220353 (JT).

"Effect of miR-15b on anthracycline-induced cardiomyocyte damage and Bcl2 protection"

Georthan Mancilla[1,2,#], Cristian Godoy[1,2], **Ingrid Oyarzún[1,2]**, Pablo Castro[1,2], Hugo Verdejo[1,2], Clara Quiroga[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. # e-mail address: endlos@ug.uchile.cl

One of the most recognized and severe long-term consequences of antineoplastic therapy corresponds to cardiac dysfunction in cancer survivors. The clinical manifestations are heterogeneous and range from asymptomatic deterioration of left ventricular ejection fraction to heart failure. Many cases are associated with the use of anthracyclines, a family of drugs widely used in cancer. Several mechanisms have been described to understand how anthracyclines contribute to cardiac damage. Signs of myocardial damage can be observed early on chemotherapy, suggesting an important role of preventive strategies to reduce it. We are interested in studying whether cardioprotection could be mediated by circulating microRNAs, and we propose that upregulation of miR-15b, an exercise-linked microRNA, prevents anthracycline-induced cardiomyocyte dysfunction. In this line, we evaluated the effect of pretreatment with the mimetic or antagonist of miR-15b on cell viability and the antiapoptotic BCL2 expression level.
Our results showed that overexpression of a mimetic miR-15b (25 nM) increased the viability of ventricular cardiomyocytes isolated from neonatal Sprague-Dawley rats and treated with 1 uM of Doxorubicin (Dox) but differ from the effect on the cancer cell line HeLa, where the overexpression of miR-15b (25 nM) increases the Dox lethality, evaluated through release LDH percentage. The transfection of antagomiR-15b also raised Bcl2 expression in cardiomyocytes at 24 hours, with partial protection against Dox treatment (6.89%).
In conclusion, our results showed a differential modulation of cell death between the cancer cells and cardiomyocytes, through a not-dependent Bcl2 mechanism, and becoming the treatment with miR-15b in a potential preventive strategy to cardiac damage derived from chemotherapy.

Fundings: This work was supported by the FONDAP-ACCDiS (15130011) and FONDECYT (1211270).

"Generation of KLK4 and KLK6 specific nanobodies for breast cancer diagnosis"

Catalina Irribarra[1], Yorka Cheuquemilla[1], Johanna Himelreichs[1], Guillermo Valenzuela-Nieto[1], Ronald Jara[1], Pamela Ehrenfeld[2], Alejandro Rojas[1]

[1] Institute of Medicine, Faculty of Medicine, Universidad Austral de Chile, Valdivia, Chile.
[2] Laboratorio de Patología, Instituto de Anatomía, Histología y Patología, Facultad de Medicina, UACh y el CISNe.

Abstract:

According to the Pan American Health Organization (PAHO), more than 462,000 women are diagnosed with breast cancer each year in the Americas, and almost 100,000 die from this disease. It is expected that by 2030 the number of women diagnosed with breast cancer will increase by 34%, mainly due to demographic changes and greater exposure to hormonal and environmental risk factors. Being the most common type of cancer in women in developed and developing countries and the second leading cause of cancer death in women worldwide, early diagnosis of this pathology is of vital importance. It has been seen that KLK4 and KLK6, kallikrein-related proteases, are proteins that act as selective biomarkers of breast cancer, therefore, the generation of tools for its study and detection is of great importance. Here, we describe the generation and characterization of alpaca nanobodies, fragments of the hypervariable region of heavy chain only antibodies present in the Camelidae family. We performed a series of immunizations, subsequent RNA extraction and bacterial display library generation, followed by selection of candidate clones using the density gradient separation protocol and high-content microscopy. After that, we validated the best candidates using KLK4-GFP and KLK6-GFP transfected cells. Obtaining specific nanobodies against KLK4 and KLK6 has great clinical significance, which, when applied, is a useful tool in the screening and early detection of breast cancer.

Funding
This work was funded by FONDECYT REGULAR 1200427 to ARF; FONIS EU-LAC T010047 to CSR, PCC, JO, JB, NLR & ARF; FONDEQUIP EQM180037 to ARF; the regional Council of the "Los Rios region" project FICR16-19, FICR19-20 and FICR21-01.

"Insulin regulates MUL1 expression in skeletal muscle cells"

Erik Lopez-Gallardo[1], Ignacio Norambuena-Soto[1], Marioly Müller-Sobarzo[1,2]
Sergio Lavandero[1,3]

[1] Advanced Center for Chronic Diseases (ACCDiS), Faculty of Chemical & Pharmaceutical Sciences & Faculty of Medicine, University of Chile, Santiago, Chile.
[2] Medical Technology Department, Faculty of Medicine, University of Chile, Santiago, Chile.
[3] Cardiology Division, University of Texas Southwestern Medical Center, Dallas, Texas, USA.

Insulin regulates mitochondrial dynamics and function by Akt, generating an elongated mitochondrial phenotype and stimulating the mitochondrial oxidative metabolism. MUL1, a mitochondrial E3 ligase, ubiquitinates Akt and Mfn2, targeting to proteasomal degradation. MUL1 also increases Drp1-induced mitochondrial fragmentation and decreases insulin-induced mitochondrial metabolism. Thus, MUL1 can regulate the insulin sensitivity. However, the role of MUL1 on the insulin signaling pathway is poorly explored. Therefore, we investigate the effect of insulin on MUL1 expression at mRNA and protein levels by RT-qPCR and western blot, respectively, in cultured rat L6 myoblasts. The results showed that the mRNA expression level of MUL1 was not altered in L6 myoblasts under insulin stimulation (10 nM). Furthermore, MUL1 protein level increased 6 hours post-stimulation with insulin, and decreased mitochondrial mass. Thus, mitophagy could be induced by an increased MUL1expression level in myoblasts treated with insulin stimulation, but further studies are required to test this hypothesis.

Funding: FONDECYT 1200490. ANID Master Fellowship 22221427. & FONDAP 15130011.

"Stress-induced cell senescence increases mitochondrial calcium uptake and induces mitochondrial dysfunction in HepG2 cells"

Lautaro Magaña[1,*], Xavier Vielma[1,*], Jose Bustamante[1], Roberto Bravo-Sagua[1,2,3]

[1] Laboratory of Obesity and Metabolism (OMEGA), Institute of Nutrition and Food Technology (INTA), University of Chile, Santiago, Chile
[2] Advanced Center for Chronic Diseases (ACCDiS), Faculty of Pharmaceutical & Chemical Sciences and Faculty of Medicine, University of Chile, Santiago, Chile.
[3] Interuniversity Center for Healthy Aging (CIES), Consortium of Universities of the State of Chile (CUECH), Santiago, Chile.

Stress-induced cell senescence characterizes by cell cycle arrest and mitochondrial dysfunction. Calcium overload is a known inducer of mitochondrial dysfunction; however, it is unknown whether ER-to-mitochondria calcium transfer participates in cell senescence. In this work, we aimed to determine mitochondrial calcium uptake from the ER changes during the development of hydrogen peroxide-induced senescence in HepG2 cells. We evaluated senescence as changes in cell morphology via light microscopy, β-galactosidase staining and expression of p16, p21 and p53 protein markers via western blot. We evaluated ER-mitochondria proximity through confocal microscopy, ER-to-mitochondria calcium transfer via live-cell fluorescence microscopy with the Rhod-FF calcium probe, and mitochondrial respiration as oxygen consumption through Clark's electrode oxygraphy. In our hands, hydrogen peroxide induced cell senescence over a time course of 3 days. During this process, ER-mitochondria proximity increased as well as mitochondrial calcium uptake from ER stores, concomitant with decreased mitochondrial respiration. In sum, our results show that ER-mitochondria physical and functional coupling increases during the course of cell senescence. Further research is necessary to determine whether these changes drive hydrogen peroxide-induced mitochondrial dysfunction.

Funding
This work was supported by National Agency of Research and Development (ANID), Chile (FONDECYT Iniciación 11201267) and University of Chile (U-Inicia UI-006/19).

"The role of METTL3 inhibitor STM2457 in cardiac fibroblasts "

Danica Jiménez-Gallegos[1,2], Sebastián Urquiza-Zurich[1,2], Sergio Lavandero[1,2], Vinicius Maracajá[1,2].

[1] Advanced Center for Chronic Diseases (ACCDiS), Universidad de Chile, Santiago, Chile.
[2] Departamento de Bioquímica, Facultad Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile. E-mail: danica.jimenez@ug.uchile.cl

Objective: STM2457 inhibitor recently described as highly specific for N6-adenosine-methyltransferase subunit (METTL3) by improving apoptosis death cell. Pathological cardiac fibrosis led to a greater ventricular stiffness of the heart with consequent heart failure. *This work evaluates the role of STM2457 in dysregulated apoptosis of activated cardiac fibroblast*. Methodology: Ventricular cardiac fibroblasts (CF) of neonatal rat were treated with TGF-β1 (10 ng/mL) for 6, 24, 48 and 72 h. Cell viability and apoptosis of STM2457 (0 – 100 μM) were evaluated by LDH assay and flow cytometry through propidium iodide and annexin V tagged cells. In a pre-treatment with STM2457, mRNA and protein expression levels of METTL3 were assessed by qRT-PCR and western blot analysis, respectively. Finally, soluble type I collagen was evaluated with picrosirius red staining from fibroblasts culture medium. Results: CF treated with TGF-β1 decreased its viability since 10 μM of STM2457 by LDH assay and increased apoptosis level with 20 μM of STM2457 by flow cytometry. Decreased METTL3 mRNA and protein levels was observed at 6 and 24h with 10 and 20 μM of STM2457. Finally, soluble type I collagen decreased at 72h. Conclusions: STM2457 improve apoptosis in cardiac fibroblasts and in consequence the reduction of the secretion extracellular matrix compound collagen type I. Relevance and projections: Zhuang et al., 2021 were the only in report the inhibition of cardiac mice fibroblasts activation by silencing METTL3. A pharmacological inhibition of METTL3 would be the first work in cardiac fibroblasts of neonatal rat, a highly used and reproducible model.

Funding: This work was supported by Asociación Nacional de Investigación y Desarrollo (ANID), Beca Doctorado Nacional 21210478 (DJG), FONDAP 15130011 (SL, VM) and FONDECYT 1211731 (VM).

"Study of TPC1-like channels in Physcomitrella patens: interaction between EF hands and Ca^{2+}"

Franko Mérida Quesada[1], Fernando Vergara Valladares[1], Carlos Navarro-Retamal[2] Ingo Dreyer[1]

[1] Laboratorio de Electrofisiología, Centro de Bioinformática, Simulación y Modelado(-CBSM), Facultad de Ingeniería, Universidad de Talca, Talca, Chile.
[2] Cell Biology and Molecular Genetics, University of Maryland, College Park, College Park, MD, United States.

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 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. However, detailed phylogenetic analyses revealed interesting aspects in mosses like *Physcomitrella patens*. In this planta branch, a second subclass of channels similar to TPC1 evolved (subclass TPC1b), which is exclusive to bryophytes. In this work, an analysis of the sequences of channels similar to TPC1 of *Physcomitrella patens* was carried out, comparing them with the sequence of TPC1 in *Arabidopsis thaliana* (AtTPC1). As a result, a total of 9 sequences belonging to the TPC1 channel family were identified in *Physcomitrella patens*. Although all them still share the structural architecture of that known from AtTPC1, the 9 proteins can be divided in two subgroups : members of one share higher identity to AtTPC1, while members of the other are less similar and can be grouped in the TPC1b subclass. In channels of this type, key amino acids that are essential for Ca^{2+} sensing (eg, in EF hands) are mutated, which could affect the channel's ability to coordinate Ca^{2+} ions. For this reason, EF hand structural models for PpTPC1 (similar to AtTPC1) and PpTPC1b channels were generated and analized in, molecular dynamics simulations.

Funding: This work was supported by the Agencia Nacional de Investigación y Desarrollo de Chile (Fondecyt No. 1220504).

"SYSTEMIX: Systems Biology Center for the study of extremophile communities from mining tailings"

Mauricio Latorre [1,2], Lorena Pizarro[3], Angélica Reyes[4], Alex Di Genova[5], Vinicius Maracaja-Coutinho[6], Valentina Parra[6*], Emilio Vilches[7]

[1] Laboratorio de Bioingeniería; Instituto de Ciencias de la Ingeniería; Universidad de O'Higgins.
[2] Laboratory of Bioinformatics and Gene Expression, INTA, University of Chile.
[3] Laboratory of Plant Immunity; Instituto de Ciencias Agroalimentarias, Animales y Ambientales; Universidad de O'Higgins.
[4] Laboratory of Microbiology and Probiotics, INTA, University of Chile.
[5] Laboratory of Computational Biology (Di Genoma Lab), Instituto de Ciencias de la Ingeniería; Universidad de O'Higgins.
[6] Advanced Center of Chronic Diseases (ACCDiS), Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas; Universidad de Chile.
[7] Laboratory of Mathematical Biology, Instituto de Ciencias de la Ingeniería; Universidad de O'Higgins.
* Research Communication Sponsor

The Cauquenes tailing located in the O'Higgins Region is, to date, the oldest and largest copper tailing reservoir of the material deposited by El Teniente. In this context, identifying and characterizing communities of extremophile microorganisms that inhabit the Cauquenes tailing will provide valuable information about the structure of these communities and how they have been maintained or changed over time. For these reasons, and through the integration of the various capacities of national and international researchers, this project seeks to establish the foundations for creating a Systems Biology Center to study the communities that inhabit mining tailings, called SYSTEMIX. At the research level, we seek for: i) Characterization of the structure of the extremophile communities; ii) Identification and validation of the metabolic potentials of the communities and their members; iii) To catalog and classify obtained information through the development of a genomic database for collected strains and; iv) Biotechnology applications. With a strong regional commitment and using a multidisciplinary and comprehensive perspective, our project will generate valuable molecular, genomic, and phenotypic information about microorganisms from extreme environments, data will be fully available for the Chilean bioinformatic community to promote new bridges of collaboration.
Funding: ANID ANILLO ACT210004.

"Structural study of the inhibitory role of naphthalene derivatives against the PLpro enzyme from the zoonotic coronavirus SARS-CoV"

Luis Castillo[1], Julio Caballero[1], José Luis Velázquez-Libera[1]

[1] Center for Bioinformatics and Molecular Simulation, Universidad de Talca, 2 Norte 685, Talca-Chile.

The protease PLpro (papain-like protease) from zoonotic coronaviruses (CoVs) is associated with different functions resulting in antagonism of the host immune response, being an attractive therapeutic target for drug design against SARS-CoV, MERS-CoV, and SARS-CoV-2. Candidate compounds derived from naphthalene have been reported to reduce the activity of this enzyme; however, few molecular modeling studies have been performed to describe their action and structure-activity relationship. To identify the structural features that facilitate the interaction between PLpro and its ligands, we performed docking of 75 naphthalene-derived inhibitors within the S3 and S4 subsites of SARS-CoV PLpro. The binding modes of the compounds were characterized in detail by chemometric analysis using the LigRMSD web server and molecular interaction fingerprints. A correlation between the docking binding free energies and the experimental activities of the studied compounds was determined by considering the flexibility of the PLpro binding site. When flexible protein structures were obtained with Gaussian Molecular Dynamics (GaMD) and clustering, a significant correlation (R2 = 0.917) was obtained. Our results show that considering the flexibility of the binding site for docking was essential to accurately describe binding events and explain why some compounds are more potent than others. Our results were obtained for SARS-CoV PLpro, but could help understand the interactions between drugs and this protease from other coronaviruses.

Funding: The assistance to the Annual Meeting of the Society for Biochemistry and Molecular Biology of Chile 2022 was supported by the Fondo Nacional de Desarrollo Cientifico y Tecnologico (FONDECYT) [1210138]. The web server is hosted in the cluster obtained with the grant [CONICYT-FONDEQUIP-EQM160063].

"Gene regulatory network of the Sulfate deficiency response in *Solanum lycopersicum*"

José D. Fernández[1,2], Jonathan Canan[1,2], Nathan R. Johnson[1,2], Diego Landaeta-Sepúlveda[1,2], Javier Canales[2,3], José M. Álvarez[2,4], Elena A. Vidal[1,2]

[1] Centro de Genómica y Bioinformática, Facultad de Ciencias, Ingeniería y Tecnología, Universidad Mayor, Santiago, Chile
[2] ANID-Millennium Science Initiative Program-Millennium Institute for Integrative Biology (iBio)
[3] Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile
[4] Centro de Biotecnología Vegetal, Facultad de Ciencias, Universidad Andrés Bello, Santiago, Chile

Sulfur (S) is an essential macronutrient and a key factor for plant growth. Deficiency of sulfate, the main source of S in soils, has profound effects on development, leading to growth retardation and a reduction in crop productivity. In tomato, sulfate deficiency has profound detrimental effects on leaf growth, that are partly explained by concomitant changes in expression of thousands of transcripts. In this work, we aim to define gene regulatory networks (GRNs) underlying this massive reprogramming of the transcriptome in leaves in response to sulfate deficiency, and to pinpoint key regulatory transcription factors. For this, we used available transcriptomic data and chromatin accessibility data of tomato leaf tissue, to determine regulatory interactions between transcription factors (TF) and their genome-wide targets. Our approach allowed us to produce a context-specific GRN for sulfate deficiency responses in tomato leaves. A rank of TFs with a central role in controlling sulfate-related genes was generated by analyzing centrality measures, as well as expression and potential involvement of TF homologs in *Arabidopsis* in nutrient responses and growth processes. The top 5 TFs are predicted to control a relevant proportion of sulfate-responsive genes in leaves, with an overrepresentation of terms related to sulfate deficiency responses, immune responses, leaf senescence and signaling pathways involving salicylic and jasmonic acid hormones. Our results provide important ground information about regulatory networks essential in tomato responses to sulfate deficiency stress.

Funding: This work was supported by ANID-FONDECYT 1211130, ANID-Millennium Science Initiative Program ICN17_022 and Beca Doctoral, Universidad Mayor.

"Identification of sulfate deficiency-responsive microRNAs in *Solanum lycopersicum*"

Diego Landaeta-Sepúlveda[1,2], Nathan R. Johnson[1,2], José D. Fernández[1,2]
Evelyn Sánchez[1,2], Gabriela M. Saavedra[1,2], Javier Canales[2,3], Elena A. Vidal[1,2]

[1] Centro de Genómica y Bioinformática, Facultad de Ciencias, Ingeniería y Tecnología, Universidad Mayor, Santiago, Chile
[2] ANID-Millennium Science Initiative Program-Millennium Institute for Integrative Biology (iBio)
[3] Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile

Sulfate is the main source of sulfur available for plants and its deficiency negatively impacts plant growth and yield. Our previous work showed that sulfate deficiency has detrimental effects on root and leaf growth in *Solanum lycopersicum*, one of the most important crops in Chile. This effect is explained in part by extensive changes in gene expression in both organs. microRNAs (miRNAs) are short non-coding RNAs with an important role in post-transcriptional regulation. We sought to determine whether sulfate deficiency triggers differential expression of miRNAs in tomato, and the impact of microRNA-dependent regulatory networks on the transcriptomic response of roots and leaves. We generated sRNA-seq libraries from roots and leaves of tomato plants grown in sulfate deficiency or control conditions for three and four weeks. De novo identification of smallRNA clusters identified 232 known expressed miRNAs in roots and leaves, of which 53 of them were differentially expressed by sulfate deficiency. Sulfate regulation of miRNAs was highly time and organ-specific, with only miR395 being consistently regulated across time points and organs. Using different bioinformatic and experimental data, we determined transcript targets for the differentially expressed miRNAs, obtaining 413 miRNA-target pairs. This information was integrated with transcription factor-target regulatory interactions to build a gene regulatory network controlled by sulfate-responsive miRNAs. This network contains genes involved in sulfate transport and metabolism, as well as other processes.

In sum our work provides evidence for a relevant role of miRNA regulation on the reprogramming of the root and leaf transcriptome during sulfate deficiency.

Funding: This work was supported by ANID-FONDECYT 1211130 and ANID-Millennium Science Initiative Program ICN17_022.

"Bioinformatic identification of genes differentially expressed and sex-related in cardiac fibrosis"

Matias Guerra[1,2], **Anita Tapia[1,2]**, Jose Miguel Ulloa[1,2], Ingrid Oyarzún[1,2], Georthan Mancilla[1,2], Pablo Castro[1,2], Hugo Verdejo[1,2], Clara Quiroga[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. # e-mail address: clquiroga@uc.cl

Patients with HFpEF are characterized by being mostly women and older than 65 years. The heart in this pathology such as other cardiovascular diseases undergoes structural changes such as hypertrophy and fibrosis that alter the normal functioning of the organ; however, the molecular mechanisms that describe the higher prevalence reported in women are still unknown. For this, one of the possible search approaches is the identification of genes differentially expressed in public transcriptomic arrays of patients with cardiovascular diseases (CVD), through a reanalysis introducing the gender approach. We propose that there are genes differentially expressed underlying to CVD that may explain the sex differences in fibrosis. Using RNA-Seq transcriptomic data obtained from the Myocardial Applied Genomics Network (MAGNet) (GSE141910), samples from 12 patients with dilated cardiomyopathy (50% women) were analyzed using different bioinformatic tools. Our results showed that in women the genes NDUFS7 (p= 1.23E-11, log2FoldChange= 2.40), COX6A2 (p= 2.37E-11, log2FoldChange= 2.81), and ATP5D (p= 1.16E-10, log2FoldChange= 2. 40) were overexpressed, while in males, the over-regulated genes were POSTN (padj= 6.05E-9, log2FoldChange= 3.09) and ASPN (padj= 3.43E-4, log2FoldChange= 1.53). To validate our findings, we use a sexed primary culture of cardiac fibroblast isolated from neonatal Sprague-Dawley rats treated with TGFβ (5 ng/ml) as a pro-fibrotic agent. The results obtained through RT-qPCR apparently validated our analysis, because showed a strong overexpression of mitochondrial genes in female fibroblast, but not in male cells.

Fundings: This work was supported by the FONDAP-ACCDiS (15130011) and FONDECYT (1211270).

"Endocytic trafficking is reprogramed in Arabidopsis root during oomycete biotrophic interaction"

Claudio Osorio-Navarro[1], Ysadora Fernandez[2], José Luis Henriquez[2], Lorena Norambuena[1]

[1]Centro de Biología Molecular Vegetal, Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile. [2]Departamento de Sanidad Vegetal, Facultad de Ciencias Agronómicas, Universidad de Chile.

Pathogen microorganisms infect host plants to acquire nutrients. Biotrophic pathogens develop specialized intracellular-feeding structures that allow the host-pathogen communication. The pathogen-induced structure is enclosed by an enlargement of the plant plasma membrane. This structure is essential for the success of pathogen proliferation where the plant endocytic trafficking machinery has been suggested plays a relevant role. We work with *Arabidopsis thaliana* and the pathogenic oomycete *Phytophthora cinnamomic* (*Pc*) to study the role of endocytic trafficking in the for plant-pathogen interaction. *Pc* proliferated through the Arabidopsis root promoting root growth inhibition; however, the aerial tissue continues growing, indicating a biotrophic interaction. The endocytic trafficking was abolished in root tip of epidermal cells after Arabidopsis-*Pc* interaction. In these conditions, the biosynthesis of the fosfoinositide $PI(4,5)P_2$ at PM was inhibited indicating that Clathrin-Mediated Endocytosis (CME) is the affected pathway. The CME inhibition occurs with independency of the place or cell type of the initial infection. More interestingly, CME is progressively inhibited over time in the root tip even though *Pc* has not reached the root tip. This strongly suggest that a mobile signal modulate CME dynamics. Consistently with the lower level of CME induced by the infection, *Pc* proliferation was faster in with CME-deficient Arabidopsis. Together, these results show that plant *Pc* invasion is associated with the inhibition of plant CME. Whether this mechanism is modulated by the pathogen for the successful infection or for the plant as a defense mechanism will be our next step of research.

Funding: FONDECYT 1211311. ANID PhD fellowship 2020-21201663.

"Boron (B) and aluminum (Al) interaction enhances physiologic parameters and antioxidant mechanism in two genotypes Al-contrasting of highbush blueberry (*Vaccinium corymbosum* L.) cultivated under acid conditions"

Katherine Salazar-Molina[1], Paz Cárcamo-Fincheira[2], Ricardo Tighe-Neira[2], Adriano Nunes-Nesi[3], Marjorie Reyes-Díaz[4], Claudio Inostroza-Blancheteau[2]

[1]Estudiante de Agronomía, Departamento de Ciencias Agropecuarias y Acuícolas, Facultad de Recursos Naturales, Universidad Católica de Temuco, Rudecindo Ortega 02950, Temuco.
[2]Departamento de Ciencias Agropecuarias y Acuícolas, Facultad de Recursos Naturales, Universidad Católica de Temuco, Rudecindo Ortega 02950, Temuco.
[3]Departamento de Biologia Vegetal, Universidade Federal de Viçosa, 36570-900 Viçosa, MG, Brazil.
[4]Departamento de Ciencias Químicas y Recursos Naturales, Facultad de Ingeniería y Ciencias, Universidad de la Frontera, Francisco Salazar 01145, Temuco.

Aluminum (Al) toxicity is a major limiting factor for the plant´s growth in acidic soils. Boron (B) is an indispensable micronutrient for plant growth. It´s reported that B could alleviate Al-toxicity and decreased oxidative stress. However, in blueberry cultivars this effect it´s unknown. The aim was evaluated the interactions B x Al on photosynthesis and secondary metabolism in two genotypes Al-contrasting of blueberry: Star (Al-sensitive) and Cargo (Al-resistant), subjected to 0 (T1), 200 (T2), 400 (T3) and 800 (T4) mg B+400 µM Al, in Hoagland solution at 4.5 pH during 72h. We evaluated photosynthesis (Pn), lipoperoxidation (LP), antioxidant activity (AA), polyphenols (PPh), and flavonoids (FV). The results showed ($p \leq 0.05$) in the factors evaluated: time, treatments, and time x treatment. LP levels was decreased significantly with B applications, in Cargo leaves under T2 and T3, and in Star to T4, while in roots alone Star genotypes decrease significantly the LP levels in T4, during 72h. PPh and FV showed in Cargo leaves an increased at 24 and 48 h in T3 and T4, and in of Star leaves the PPh were increased at 48 and 72h in T4. Meanwhile, the B applications enhanced Pn significantly in Star at 48 and 72h under T3 and T4, and Cargo in T2 during all times. In conclusion, its suggested that B-applied induce in roots and leaves of the cultivar Al-sensitive an increase antioxidant mechanism and Pn under Al-stress, in the same way, showed an enhance in antioxidant response of the Al-resistant genotypes.
Keywords: Aluminum toxicity, antioxidant activity, lipoperoxidation, polyphenols.

Acknowledgment: FONDECYT Regular 1201749, 1211856 and 3220674 projects.

"Characterization of glycogen phosphorylase activity in the absence of the pyridoxal-5'-phosphate cofactor"

Nicolás Herrera-Soto[1], Felipe González-Órdenes[1], Ernesto Uribe-Oporto[1], Victor Castro-Fernández[1], Victoria Guixé[1*]
*Research Communication Sponsor

[1] Laboratorio de Bioquímica y Biología Molecular, Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile.

Glycogen phosphorylase (GP) catalyzes the rate-limiting step in glycogenolysis, releasing glucose-1-phosphate from the terminal alpha-1,4-glycosidic bond through a mechanism that involves the cofactor pyridoxal-5'-phosphate (PLP). In all GP reported to date the cofactor is covalently linked to a strictly conserved lysine residue of the active site through a Schiff base. However, multiple sequence alignment of GP from the three domains of life indicated that the lysine involved in PLP binding is replaced by a threonine in the *Methanococcales* group of archaea. To characterize the kinetic features of enzymes from this group, we chose the GP from the archaea *Methanococcus maripaludis* as a model. Fluorescence and mass spectrometry analysis indicated the absence of the cofactor PLP in the MmGP enzyme. However, the enzyme is active and show preference by for large and ramified substrates like glycogen. The role of the threonine residue in enzyme catalysis and PLP binding was evaluated by site-directed mutagenesis replacing the residue by lysine (T448K).
The mutant displays a 30-fold reduction in the enzyme activity and a partial recovery of PLP binding as indicated by the fluorescence emission peak at 530 nm. The results demonstrate for the first time that phosphorylase activity is possible in the absence of PLP and highlights the requirement of further site directed mutagenesis to identify residues involved in catalysis.

Funding: Fondecyt 1191321

"The long non-coding RNA MALAT1 modulates a novel downstream regulatory element of NR4A1 gene in specific cancer-type cells"

Rodrigo Maldonado[1], Sara Wernig-Zorc [2], Uwe Schwartz[3], Josefa Inalef [1], Francisca Pavicic[4], Pamela Ehrenfeld[4], Gernot Längst [5], Rodrigo Maldonado[1]

[1] Laboratory of RNA and Epigenetics, Instituto de Anatomía, Histología y Patología, Facultad de Medicina, Universidad Austral de Chile, Valdivia, Chile.
[2] St. Anna Children's Cancer Research Institute, Vienna, Austria.
[3] NGS Analysis Center, Biology and Preclinical Medicine Faculty, University of Regensburg.
[4] Laboratorio de Patología Molecular, Instituto de Anatomía, Histología y Patología, Facultad de Medicina, Universidad Austral de Chile, Valdivia, Chile
[5] Biochemistry III Institute, Biology and Preclinical Medicine Faculty, University of Regensburg.

Long non-coding RNAs (lncRNAs) act as transcriptional regulators in Cis or Trans, depending on their functional interaction with chromatin and chromatin-associated factors on a cell-specific manner. Therefore, alterations on the expression and activities of lncRNAs have been vastly associated with the development of specific tumorigenic diseases. Nevertheless, the molecular mechanisms of how lncRNAs can directly-define the transcriptional levels of target genes is still a matter of debate. In this work, we knocked-down (KD) the chromatin-associated lncRNA MALAT1 to assess the transcriptome (RNA-seq) and chromatin accessibility (ATAC-seq) effects at two different timepoints to define directs target genes. The results show that MALAT1 directly modulate a define number of coding genes, where significant accessibility changes were observed. Next, using the CrispR-Cas9 technology we showed that MALAT1 modulates the accessibility of a downstream regulatory element of the NR4A1 gene, to fine-tune its expression. Bioinformatic analyzes using TCGA data, revealed a direct correlation for NR4A1/MALAT1 expression concomitant with the accessibility of the downstream regulatory element on specific cancer types, like breast cancer. Accordingly, the analysis of this molecular mechanism on breast cancer cells (MCF7) compared to another cancer cell type (Pancreatic Duct Epithelioid Carcinoma, PANC1) that do not show any correlation for the NR4A1/MALAT1 expression and the NR4A1 downstream element axis, demonstrates that MALAT1 regulates the expression of NR4A1 cell specifically.
Our results delineate the MALAT1 molecular mechanism to fine-tune the expression of specific cancer drivers like NR4A1 in breast cancer.
Funding: This work was supported by the Comisión Nacional de Investigación Científica y Tecnológica (CONICYT), through an Inititation in Science FONDECYT grant number 11190246.

"The long non-coding RNAs CRNDE and MIAT are potentially involved in the altered cardiac differentiation of iPSCs from Down Syndrome patients"

Francisco Sigcho[1,2,3], Wladimir Corrales[2,3,4], Leslye Venegas[1,2], Sebastián Leiva-Navarrete[1,2,3,5], Vinicius Maracaja-Coutinho[1,2,3,5], Valentina Parra[1,2,5]

[1] Advanced Center of Chronic Diseases (ACCDiS), Facultad de Ciencias Químicas y Farmacéuticas y Facultad de Medicina, Universidad de Chile, Santiago, Chile.
[2] Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile.
[3] Centro de Modelamiento Molecular, Biofísica y Bioinformática (CM2B2), Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile.
[4] Laboratorio de Neuroplasticidad y Neurogenética. Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile.
[5] Systems biology center for the study of extremophile communities from mining tailings (SYSTEMIX), O'Higgins University, Rancagua, Chile.

Down syndrome (DS) is characterized by a genetic imbalance that disturbs the transcriptome and various cellular processes, such as cardiogenesis, increasing the risk of several pathologies including congenital heart diseases. Long non-coding RNAs (lncRNAs) can act as important regulators of cardiogenesis. Additionally, cardiac differentiation in cells derived from DS patients (3S) is deficient compared to non-trisomic individuals (2S). Here, we hypothesize that "lncRNAs associated with cardiogenesis are differentially expressed (DE) in induced pluripotent stem cells (iPSC) from DS patients". To evaluate this hypothesis, we performed a meta-analysis of transcriptome datasets focusing specifically on lncRNAs. First, a database search was performed in GEO, using the keywords: iPSCs/trisomy/Homo sapiens. Then, three microarray and three RNA-seq datasets that compared 3S/2S iPSCs profile expression were selected. Next, 3S/2S DE lncRNAs were determined using GEO2R and DeSeq2 for microarray and RNA-seq datasets, respectively (p-value<0.05, FC>1.5).

Heatmap and Upset plot were used to identify the DE lncRNAs that could be affecting 3S iPSCs cardiac differentiation. Then, we measured the transcript levels of lncRNAs in 2S and 3S iPSC by qPCR. Additionally, we also standardized the differentiation of 2S and 3S iPSCs into cardiomyocytes. We found that MIAT and CRNDE are 3S/2S DE lncRNAs, which were overexpressed in 3S iPSCs. Also, we demonstrated that 3S iPSCs differentiation into cardiomyocytes was altered. Finally, we suggest that CRNDE and MIAT are lncRNAs involved in the decreased cardiomyocyte differentiation of 3S iPSCs. Our next goal is to characterize the mechanism of these lncRNAs and their role in DS cardiomyocyte differentiation.

Funding: This project is funded by ANID FONDECYT 1190743 (VP), 1211731 (VM-C) and FONDAP 15130011 (VP, VM-C); Universidad de Chile grants Enlace FONDECYT I0230/2020 (VM-C) and Apoyo a la Infraestructura para la Investigación INFRA-021/01/2019 (VM-C); ICGEB CRP/CHL18-04 (VP), and ANID PhD scholarship 21210841 (FS).

"Comparative analysis of the effect of General Regulatory Factors on ISW1a Nucleosome Remodeling Activity"

Andrea Oyarzún[1], Roberto Amigo[1], Cristián Gidi[1], José Gutiérrez[1]

[1] Laboratorio de Regulación Transcripcional, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.

In chromatin, nucleosome arrays are often interrupted by Nucleosome Depleted Regions (NDRs), present in transcriptional regulatory regions and other genomic regions. Several Chromatin Remodeler Complexes play roles in NDR formation and maintenance. For instance, ISW1a slides nucleosomes towards NDRs. These regions are occupied by protein complexes. In budding yeast, this complex could contain General Regulatory Factors (GRFs). Their occupancy antagonizes nucleosome formation at NDRs. GRFs could act as barriers to the activity of remodelers such as ISW1a, but their individual ability to do this has not been directly tested. Thus, we performed a comparative analysis of the effects of the main GRFs (Reb1, Abf1, Cbf1 and Rap1) on nucleosome sliding by ISW1a.
His-tag GRFs and ISW1a were purified from *E. coli* BL-21 and *Saccharomyces cerevisiae* YFR013w, respectively. DNA probes harboring the 601 sequence and binding sites for each GRF were labeled with ^{32}P-γ-ATP to generate reconstituted mononucleosomes. Remodeling assays were performed using these components and were analyzed by electrophoresis on native polyacrylamide gels.
ISW1a was efficiently blocked by Rap1. Abf1 displayed a weaker blocking effect. There were no significant differences using Reb1 or Cbf1. Further analysis show a correlation between these results and affinity and dissociation kinetics analysis. Afterwards, additional comparative analysis, using optimized removal of Rap1 after remodeling, confirmed that Rap1 effectively blocks ISW1a sliding activity. These results suggest that the high affinity of Rap1 to its target sequence plus its slow dissociation kinetics conform the underlying mechanism giving to Rap1 a strong ability to stop ISW1a sliding activity.

Funding: This work was supported by ANID/FONDECYT Regular 1180911.

"Effect of poly (dA:dT) tracts on nucleosome remodeling activity of ISW1a and RSC complexes"

Roberto Amigo[1], José Leonardo Gutiérrez[1]

[1]Laboratorio de Regulación Transcripcional, Departamento Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción

Nucleosome positioning is crucial for cellular processes that involve protein-DNA interactions, as in the case of transcription. Gene promoters in Saccharomyces cerevisiae are highly enriched in homopolymeric DNA sequences called poly (dA:dT) tracts. These sequences are correlated with the presence of nucleosome-depleted regions (NDRs). In organisms like human and mouse, the presence of poly (dA:dT) tracts is lower and their distribution is wider; however, their ability to exclude nucleosomes is still present.
How are NDRs around poly (dA:dT) tracts formed and maintained? Genome wide analyses have found that ATP-dependent chromatin-remodeling complexes are key in these processes. In addition, these studies have shown a correlation between the action of remodeler complexes and the presence of poly (dA:dT) tracts. Nevertheless, the molecular mechanisms are still unknown.
In this context, our goal was to determine the influence of poly (dA:dT) tracts on the activity of ATP-dependent chromatin-remodeling complexes. We chose RSC and ISW1a complexes given their antagonistic roles at NDRs. We found that poly (dA:dT) tracts can influence the action of these complexes in several aspects, such as: binding to nucleosome, strength of remodeling and the direction in which the histone octamer is moved. In addition, we observed a differential effect on RSC and ISW1a activities. In summary, our results highlight the role of poly (dA:dT) tracts on nucleosome positioning and elucidate molecular mechanisms by which these sequences exert their effects.

This work was supported by ANID/FONDECYT Regular 1180911.

"Functional evaluation of N- versus C-terminal orientation of optogenetic switch components"

Sebastián Dehnhardt-Amengual[1,2], Leonardo Guzmán-Zamora[1,2], Vicente Rojas[1,2], Luis F. Larrondo[1,2].

[1]Pontificia Universidad Católica de Chile, Facultad de Ciencias Biológicas.
[2]Instituto Milenio de Biología Integrativa (iBio).

The yeast two-hybrid assay (Y2H) has been developed as a simple molecular technique capable of unveiling protein-protein interaction by reconstituting a GAL4 functional transcription factor. Although this tool is still considered relevant due to its simplicity, it is not exempt from certain restrictions in its operation, especially when the order of fusion proteins, i.e., their N- or C-terminal position, is considered.

Fungal-Light Oxygen Voltage (FUN-LOV) is an optogenetic switch based on a Y2H architecture, combining light-sensing domains and GAL4 moieties. In yeast cells, FUN-LOV shows a high dynamic range of gene expression in response to blue-light while exhibiting low basal levels of expression in darkness. The modularity of this switch has been explored exchanging its elements by others of similar nature. However, little is known about this same modularity in terms of the order of its core domains. In this work we tested the functionality of FUN-LOV by assessing variants that alter the fusion protein order from N- to C-terminal.

In order to do this, new genetic constructs were generated through PCR amplification steps and in vivo yeast homologous recombination, to then evaluate them under different illumination conditions. In contrast with the great performance when FUN-LOV photoreceptors domains are at the N-terminus of each switch component, we observed no response to light in variants with C-terminal arrangement. Further analyses confirmed that maintaining the LOV moiety N-terminal of the GAL4 DNA-Binding Domain, is the most critical variable to achieve functionality. Indeed, in silico modelling predicts a possible conformational stress of the C-terminal variant. We are currently exploring whether the use of long flexible linkers may allow the successful development of the latter type of variant.

Funding by FONDECYT 1211715 and iBio.

"Characterization of non-coding RNAs in response to copper in E*nterococcus faecalis*"

Jorge Torres [1], Sebastián Gómez [1], Victor Aliaga-Tobar [1], Mauricio Latorre [1,2,3]

[1] Bioengineering Laboratory; Institute of Engineering Sciences; O'Higgins University, Rancagua, Chile.
[2] Laboratory of Bioinformatics and Gene Expression, INTA, University of Chile, Santiago, Chile.
[3] Systems biology center for the study of extremophile communities from mining tailings (SYSTEMIX), O'Higgins University, Rancagua, Chile.

In *Enterococcus faecalis*, intracellular copper levels are regulated by the cop operon. Additionally, when exposed to Cu, a significant change is observed in this bacterium at the global transcriptional level, suggesting the presence of other regulatory mechanisms involved in the response. Non-coding RNAs (ncRNAs) correspond to a family of regulatory RNA molecules. To date, around 230 ncRNAs have been predicted in *E. faecalis*. Given this, the present work was aimed at identifying ncRNAs induced in response to Cu in *E. faecalis*. First, for a total of 585 possible ncRNAs encoded in this bacteria, 1,804 potential regulatory targets were identified using the IntaRNA software. Subsequently, after applying the selection criteria, a final list composed of 4 ncRNAs and 4 transcriptional targets was obtained. Subsequently, possible changes in the abundance of these 6 elements were studied against a non-lethal Cu exposure by means of qPCR. As a result, it was obtained that ncRNA_1959 and sRNA_069_5_UTR increased approximately 8 and 4 times, respectively, their transcriptional abundance in the Cu-supplemented condition compared to the basal condition, an expression that positively correlated with their regulated targets, suggesting a possible positive regulation to post-transcriptional level by these ncRNAs. On the contrary, the ncRNA_1490 decreases its expression by half in response to copper concerning the basal condition, negatively correlating with the expression of its target gene. Finally, the results of this thesis allowed us to identify for the first time a set of ncRNAs in response to Cu in a bacterial species.

Funding: ANID FONDECYT 1190742 (ML), ANID BASAL FB210005 (ML), ANID MILENIO ICN2021_044 (ML), ANID ANILLO ACT210004 (ML) and ACT20210044 (ML), ANID Postdoctoral 3220080 (VAT).

"Identification of non-coding RNAs in response to copper and antibiotics in *Enterococcus faecalis*"

Javiera Pino [1], Victor Aliaga-Tobar [1], Fernanda Fredericksen[1]
Mauricio Latorre [1,2,3]

[1] Bioengineering Laboratory; Institute of Engineering Sciences; O'Higgins University, Rancagua, Chile.
[2] Laboratory of Bioinformatics and Gene Expression, INTA, University of Chile, Santiago, Chile.
[3] Systems biology center for the study of extremophile communities from mining tailings (SYSTEMIX), O'Higgins University, Rancagua, Chile.

Enterococcus faecalis is a bacterium of great interest due to its high ability for resistance to antibiotics and antimicrobials, producing outbreaks of nosocomial infections inside healthcare centers. The control of this bacterium is a challenge because of the mechanisms used for its survival; not only its antibiotic resistance has been demonstrated, but also to heavy metals with antimicrobial properties such as copper. One of the regulatory elements that can be involved in antimicrobial resistance are the non-coding RNAs (ncRNAs), responding in their expression when these bacteria are exposed to a stressor. To demonstrate this, two steps were necessary to observe if *E. faecalis* responds to an antimicrobial, increasing the relative expression of a ncRNA: 1) In silico analysis of massive data of RNAseq and identification of ncRNA candidates, and 2) In vitro validation using RT-qPCR of the ncRNAs identified in response to the exposure of one antibiotic or copper. A total of 3 ncRNAs were identified, differentially expressed when the bacterium was exposed to one or more stressors. Interestingly, some of the ncRNAs are expressed in both copper and antibiotics, which could mean that the mechanism of response might be related. These results are statistically significant, so continuing with this study allows to have an approach to a new antibiotic therapy to achieve effective control of the bacterium.

Funding: ANID FONDECYT 1190742 (ML), ANID BASAL FB210005 (ML), ANID MILENIO ICN2021_044 (ML), ANID ANILLO ACT210004 (ML) and ACT20210044 (ML), ANID Postdoctoral 3220251 (FF) and 3220080 (VAT).

"Genomic characterization of the gene encoding for the APX1 enzyme in octoploid strawberry (*Fragaria* × *ananassa*) and its tissue-associated expression pattern"

Vanessa Gonzalez-Garrido[1,2], Paz E. Zuñiga[2,3], Karla Jara-Cornejo[2,3], Claudia Rivera-Mora[2,3], Carlos R. Figueroa[2,3]

[1] School of Biochemistry, Institute of Biological Sciences, Universidad de Talca, Talca, Chile.
[2] Laboratory of Plant Molecular Physiology, Institute of Biological Sciences, Universidad de Talca, Chile.
[3] Millenium Nucleus for the Development of Super Adaptable Plants (MN-SAP), Santiago, Chile.

Currently, oxidative stress in plants occurs as a direct consequence of biotic and abiotic factors, evidenced by the increase in reactive oxygen species (ROS) that leads, at the same time, to the increase in the activity of related enzymes such as ascorbate peroxidase enzyme (APX). In the *Fragaria* × *ananassa* fruit, the activity of this enzyme increased after exogenous application of methyl jasmonate suggesting a probable transcriptional regulation mediated by phytohormones. In the present investigation, we identified and characterized in silico the APX1 gene of *F.* × *ananassa*, especially its genomic organization and promoter region using the 'Camarosa' v1.a2 genome and the Blast tool. For the analysis of promoter's cis-elements, the PlantPAN and newPlace platforms along with literature-based manual curation were used to identify those that respond to various phytohormones. Phylogeny studies and homology modeling were performed using the MEGA and MODELLER software, respectively. Gene expression was also evaluated in published transcriptome data of different developmental stages of receptacle and achene. Four homologous copies for FaAPX1 and its regulatory elements for various phytohormones were identified. Three-dimensional modeling showed 86% identity with our study sequences, characterized by the presence of two beta-sheet type structures, ten alpha-helix, and one helix 3.10. The different copies of the FaAPX1 gene showed a strong expression from green to ripe stages. The results obtained are projected to improve the available information and possible in vivo applications to clarify the role of FaAPX1 and its relationship to plant hormones.

Funding: National Research and Development Agency (ANID, Chile) through FONDECYT/Regular 1210941, Millenium Science Initiative Program - NCN2021_010, and BECAS/DOCTORADO NACIONAL/2020 21201520, 21201418, and 21190862.

"Expression of Calmodulin-binding Transcription Activator (CAMTA) genes during strawberry (*Fragaria x ananassa*) fruit development"

Claudia Rivera-Mora[1,2], Paz E. Zúñiga[1,2], Karla Jara-Cornejo[1,2], Mario Moya[1,2], Lida Fuentes-Viveros[3], Carlos R. Figueroa[1,2]

[1]Laboratory of Plant Molecular Physiology, Institute of Biological Sciences, Universidad de Talca, Talca, Chile.
[2]Millenium Nucleus for the Development of Super Adaptable Plants (MN-SAP), Santiago, Chile.
[3]Centro Regional de Estudios en Alimentos Saludables (CREAS), CONICYT-Regional GORE Valparaíso Proyecto R17A10001, Valparaíso, Chile.

The commercial strawberry (*Fragaria* × *ananassa*) is a species of economic interest and is considered a model species for fruit ripening studies. The 'Calmodulin-binding Transcription Activator' (CAMTA) is a family of transcription factors present in multicellular eukaryotic organisms and is characterized by being regulated by calmodulin, the most studied calcium-sensing protein of calcium signaling. It has been related to development, growth, and stress tolerance in plants, but their participation during fruit ripening is unknown. Therefore, the main objective of this work is to identify the CAMTA genes in the *F. ananassa* genome and to analyze their expression during fruit development. A preliminary analysis of the genome of Fragaria vesca, a subgenome of *F.* × *ananassa*, indicates that it has four CAMTA genes being their expression evaluated in young and mature fruit developmental stages. Thirteen FaCAMTA genes were identified in the *F.* × *ananassa* genome and analysis of previously published transcriptomic data, from two fruit tissues (achene and receptacle) at four developmental stages, indicates that all FaCAMTA genes are expressed in both tissues at all stages. In general, the FaCAMTA genes have a higher expression in the green fruit stage and the FaCAMTA3-1 and FaCAMTA5-3 genes showed a higher expression level in both tissues, decreasing towards the end of ripening. The FaCAMTA1-4 gene exhibited the lowest expression level in all stages. Relative expression analysis by RT-qPCR of whole fruits in five developmental stages indicates similar results. These results provide valuable information for the functional analysis of CAMTA transcription factors during strawberry fruit ripening.

Funding: National Research and Development Agency (ANID, Chile) through BECAS/DOCTORADO NACIONAL (2019/21190862), (2020/21201520), and (2020/21201418); FONDECYT/Regular (1201662 and 1210941); CONICYT-Regional GORE Valparaiso (R17A10001); and Millenium Science Initiative Program (NCN2021_010).

"SALL2 expression in colon cancer progression and its association with the WNT/β-catenin pathway"

Aracelly Quiroz[1], Constanza Mardones[1], José Miguel Navarrete[1], Ángela Ortíz[1]
Paula Medina[1], Víctor Fica[2], Alexis Salas[2], Carolina Delgado[3], Ariel Castro[1]
Roxana Pincheira[1].

[1] Laboratorio de Transducción de señales y Cáncer, departamento de Bioquímica y Biología Molecular, Universidad de Concepción.
[2] Laboratorio de Diseño Racional de Fármacos, departamento de Farmacología, Universidad de Concepción.
[3] Unidad de Anatomía Patológica, Hospital Guillermo Grant Benavente.

SALL2 is a developmental transcription factor involved in the regulation of cell proliferation, migration and survival. Massive analyses indicated that *SALL2* mRNA significantly decreases in colorectal cancer (CRC), a cancer type characterized by hyperactivation of the WNT pathway. Interestingly, our unpublished ChIP-seq data analyses suggested that SALL2 regulates genes associated with the WNT pathway. However, the role of SALL2 in CRC and its relationship with the WNT pathway are yet unknown. We used tissue microarray of 130 samples from Guillermo Gantt Benavente's Hospital comprising normal colon, adenoma, and CRC to evaluate the expression and cellular location of SALL2 using chromogenic immunohistochemistry and multiplexed immunofluorescence. We found that SALL2 expresses in the nucleus and cytoplasm of the normal colon epithelium and the stroma. However, its expression decreases in adenoma and is absent in CRC. We noted a negative correlation between SALL2 expression and the expression of nuclear β-catenin, at the migratory front. Subcellular fractionation and immunofluorescence experiments in CRC cells with gain and loss of SALL2 function also support the negative correlation between SALL2 expression and nuclear β-catenin. Additionally, we evaluated the expression of WNT targets by Western blot and qRT-PCR, finding a positive correlation between SALL2 and AXIN2, a negative regulator of the WNT pathway. Analysis of *AXIN2* promoter identified several putative SALL2 binding sites, and SALL2 increases *AXIN2* promoter activity, suggesting a SALL2- dependent transcriptional regulation. Besides, we confirm that SALL2 binds to *AXIN2* promoter, using ChIP-qPCR.
Finally, we show that the SALL2-AXIN2 axis is required for the survival response to XAV939, an inhibitor of the Wnt pathway. Our study shows that SALL2 positively regulates the transcription of AXIN2. We proposed that the loss of SALL2 expression during CRC progression contributes to the decrease of AXIN2 levels, the hyperactivation of the WTN pathway, and the malignant phenotype.
Funding: FONDECYT grants 1191172 and 1201215

Modulation of pre-vacuolar traffic mediated by the MON1/CCZ1 complex in roots increases vacuolar sodium accumulation capacity and tolerance to salt stress in *Arabidopsis thaliana.*

José Madrid-Espinoza[1], Josselyn Salinas-Cornejo[1], Ricardo Pérez-Díaz[1], Simón Ruiz-Lara[1]

[1]Laboratorio Genómica Funcional, Instituto de Ciencias Biológicas, Universidad de Talca, Talca, Chile.

Endocytosis and pre-vacuolar vesicle trafficking perform essential functions in the plant cell, such as the internalization and mobilization of proteins and membranes between organelles. One of the main routes is the pre-vacuolar pathway that plays an important role in the salt stress tolerance. The final step of this pathway is regulated by the MON1/CCZ1 (MONENSIN SENSITIVITY1/CALCIUM CAFFEINE ZINC SENSITIVITY1) complex, that act as a GEF (Guanine Nucleotide Exchange Factor) protein activating RabG proteins and directing vesicle trafficking between the late endosome and the tonoplast. During salt stress, the vacuole actively participates in the accumulation of sodium, preventing its mobilization to the aerial organs and avoiding the increase in the ionic strength of the cytoplasm. Here, we show that co-expression of *SchMON1/SchCCZ1* driven by the root-specific promoter, *pUGT* (pRoot), increases vacuolar sodium content, resulting in increased tolerance to salt stress in *Arabidopsis thaliana*. The rescue of the phenotype of the *mon1-1* and *ccz1a/b* mutants, as well as sub-cellular localization studies, confirm the functionality of both proteins. Co-expressing plant lines exhibited improved cell function, with lower H2O2 production, and increased endocytic and autophagic activity, and decreases sensitivity to the application of exogenous ABA exogenous ABA. Interestingly, an RT-qPCR-based analysis on these plants showed a strong up-regulation of response genes associated with tolerance mechanisms during salt stress, suggesting that MON1/CCZ1-mediated root modulation of pre-vacuolar traffic in the roots could be used in plant breeding programs.

Funding: This work was supported by the Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT) Grant No. 1211180; the CONICYT-CHILE doctoral fellowships Grant No. 21170702.

"Differential Expression of Arabinogalactan Proteins (AGPs) In Response to Inclination in Stem of *Pinus radiata* Seedlings"

Tamara Méndez[1], Yazmina Stappung[1], Rodrigo Retamal[1], María A. Moya-León[1] Raúl Herrera[1]

[1]Instituto de Ciencias Biológicas, Universidad de Talca. Av. Lircay s/n. Post Code 3465548 Talca, Chile.

Arabinogalactan proteins (AGPs) are members of a protein family that play important roles in cell wall dynamics. AGPs presences from inclined pines were determined using JIM7, LM2, and LM6 antibodies, showing a higher concentration in one stem side. The accumulation of AGPs in xylem and cell wall tissues is enhanced in response to loss of tree stem verticality. The differential gene expression of AGPs indicates that these proteins could be involved in the early response to inclination and, trigger signals such as lignin accumulation, as well as thickening cell wall and lamella media to restore stem vertical growth. A subfamily member of AGPs, which is Fasciclin-like has been described in angiosperms as inducing due to tension wood, but also has been described in some gymnosperms as response to compression wood. A search for gene sequences of this subfamily was performed on an RNA-seq library, where 12 sequences were identified containing one or two fasciclin I domains (FAS), named PrFLA1 to PrFLA12. Four of these sequences were phylogenetically classified in group A, where PrFLA1 and PrFLA4 are differentially expressed in tilted pine trees.

Funding: This work was supported by ANID-Fondecyt (number 1201011).

"NUAK1 positively regulates nuclear PFKP levels in breast cancer cells"

Luis Espinoza[1], Pablo Parra[2], Mario Palma[1], 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.

The platelet isoform of phosphofructokinase-1 (PFKP) is a cytosolic and rate-limiting glycolytic enzyme found overexpressed in several types of cancer. PFKP also localizes in the cell nucleus, functioning as a transcriptional coactivator of important regulators of gene expression, such as c-myc and YAP/TAZ. CDK6 inhibits PFKP enzymatic activity through phosphorylation and inhibition of PFKP tetramerization. Consequently, PFKP forms a dimer, exposing a nuclear localization sequence (NLS) that drives its nuclear translocation. In breast cancer cells, we found that NUAK1, a 75kDa serine/threonine kinase member of the AMPK family associated with poor prognosis in cancer, co-immunoprecipitated with PFKP. Like CDK6, NUAK1 inhibited PFKP enzymatic activity, demonstrated by shRNA-dependent NUAK1 depletion or inhibition with HTH-01-015, a specific NUAK1 inhibitor. Based on these results, we evaluated NUAK1's role in the subcellular localization of PFKP. By immunocytochemistry and western blotting, we demonstrated that NUAK1 increases PFKP levels in the nucleus of the MCF-7 breast cancer cells. Likewise, HTH-01-015 decreased nuclear PFKP levels. Bioinformatics analysis predicted putative PFKP phosphorylation residues for NUAK1, highlighting threonine-313 in the oligomerization interface of the dimer. Our findings suggest that NUAK1 regulates PFKP enzymatic activity and promotes its nuclear localization, which may contribute to PFKP´s role in breast cancer cell-associated gene expression.

Funding: This work was supported by the National Fund for Scientific and Technological Development (FONDECYT Regular: 1201215 and 1191172).

"Understanding MYC function through its interaction with NSD3S"

Valentina González-Pecchi[1], Mario Palma Alarcón[2]

[1] Laboratorio de Investigación en Ciencias Biomédicas, Departamento de Ciencias Básicas, Facultad de Medicina, Universidad Católica de la Santísima Concepción, Concepción, Chile
[2] Laboratorio de Transducción de señales, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile

MYC is an oncogene, a transcriptional regulator and a well validated cancer drug target. However, targeting MYC has been challenging. Because MYC function is controlled by signaling proteins, one approach is to discover critical regulators of MYC and inhibit MYC using protein-protein interactions (PPI) disruptors. We identified a novel MYC partner, an epigenetic regulator, NSD3S which is amplified in multiple cancers and functions as an oncogene. We demonstrated that NSD3S interacts with MYC under physiological conditions in cancer cells. We defined the interaction interface between a required 15-amino acid region on NSD3S and MYC, as NSD3SΔ15 (NSD3S full length without those 15-amino acid) does not bind to MYC. Overexpression of NSD3S increases MYC protein stability and transcriptional activity. Mechanistically, NSD3S binds to MYC and reduces the association of F-box and WD repeat domain containing 7 (FBXW7) with MYC, which results in suppression of FBXW7-mediated proteasomal degradation of MYC and an in increase MYC protein half-life. We generated cancer cell lines expressing either NSD3S or NSD3SΔ15, having different phenotypic profiles. Interestingly, the NSD3S have a more oncogenic phenotype, agreeing in an oncogenic interface between these two proteins. These results support a critical role for NSD3S in the regulation of MYC function and provide a novel mechanism for NSD3S oncogenic function through inhibition of FBXW7- mediated degradation of MYC. The study suggests a novel regulatory axis between NSD3S and MYC and a novel therapeutic approach for treating patients with MYC-driven tumors.

Funding: This work was supported by ANID (PAI77200098).

"A novel treatment combined with a chemokine receptor inhibitor sensitize the cisplatin-resistant gastric cancer cells"

María Reyes[1], Bárbara Mora[2], Carmen Ili[1], Yuselin Mora[1], Kurt Buchegger[3], Ismael Riquelme[2], Louise Zanella[1], Priscilla Brebi[1*].

[1] Millennium Institute on Immunology and Immunotherapy. Laboratory of Integrative Biology (LIBi), Centro de Excelencia en Medicina Traslacional (CEMT), Scientific and Technological Bioresource Nucleus (BIOREN), Universidad de La Frontera, Temuco, Chile
[2] Instituto de Ciencias Biomédicas, Facultad de Ciencias de la Salud, Universidad Autónoma de Chile, Temuco, Chile
[3] Centro de Ciencias Biomédicas y Morfofuncionales (CEBIM), Department of Basic Sciences, School of Medicine, Universidad de La Frontera, Temuco, Chile

Gastric cancer (GC) is one of the leading cancer-related causes of death worldwide, emphasizing Chile. Cisplatin (CDDP) is the most commonly used drug in the chemotherapy regimen for advanced GC. Unfortunately, the high recurrence rate of GC is predominantly attributable to chemoresistance. The CCR5/CCL5 axis, that participates in inflammatory process, has been associated with the development and progression of cancer. However, its role in GC chemoresistance has not been fully elucidated. This study aimed to determine the effects of the blockade of the CCR5/CCL5 axis by a chemokine receptor inhibitor (CRI), on AGS cells (a human gastric adenocarcinoma cell-line) resistant to CDDP (AGS R-CDDP).
Methodology: AGS R-CDDP cells were obtained based on a stepwise dosing drug protocol. CCL5 candidate was selected through transcriptomic analysis, and the CCL5 expression level was validated by qRT-PCR. Flow cytometry techniques were applied to evaluate the induction of apoptosis and cell cycle. The cytotoxicity was determined by MTT assays. CRI was used alone and in combination with CDDP in all assays.
Results and conclusions: The cytotoxicity assays showed that CRI/CDDP combination re-sensitized AGS R-CDDP cells, decreasing cell viability, on the other hand, not increasing apoptosis. After 48 hours of treatment, the cell cycle assay revealed cells mainly arrested in S Phase. CCL5 showed a decrease in mRNA levels after the CRI/CDDP combination, possibly correlating with allosteric inhibition of the CCR5 receptor. Our results indicate that CRI/CDDP combination sensitized AGS R-CDDP cells to CDDP treatment revealing it is potential coadjuvant in GC therapy.

This work was supported by National FONDECYT projects (3210629, 1210440, 3210687), National FONDEF projects (VIU 20P0023, FONDEF Idea ID21I10027); Millennium Institute on Immunology and Immunotherapy (IMII) (ICN2021_045); and Internal project (DIUAV 03-2022).

"A simple method for the identification of nanobodies by density gradient separation"

Johanna Himelreichs[1], Guillermo Valenzuela-Nieto[1], Ronald Jara[1], Alejandro Rojas[1,2]

[1] Institute of Medicine, Universidad Austral de Chile, Valdivia, Chile
[2] Berking Biotechnology, Valdivia, Chile

Contact: johimelreichs@gmail.com

Camelids IgG2 and IgG3 antibodies lack interaction with light chains, thus, they are known as single chain antibodies. Regardless the simplicity of the molecules, single chain antibodies are highly effective antibodies. The hypervariable region of single chain antibodies are known as Vhh, or Nanobodies. These molecules have multiple advantages over conventional antibodies, considered an excellent biotechnological tool for multiple applications.
Our objective is to use the extraordinary nature of the camelid immune system to generate immunotherapies and diagnostic tools against various emerging infectious diseases. Today, our technological platform is able to exploit the biotechnological potential of our country, directing the immune response of alpacas to generate targeting nanobodies related to diseases of interest. From a small blood sample, we obtain peripheric lymphocytes as a representation of the alpaca's immune system, and further mRNA is isolated, transformed into cDNA and introduced into a bacteria display system for selection. Here, we generated a simple, inexpensive, and highly efficient protocol in the search for nanobodies using gradient centrifugation. Our technological platform can identify subnanomolar affinity nanobodies against important human or veterinarian diseases in a quick, efficient, easy, and economic manner, aiming for the generation of measures that can actively contribute to future pandemic preparedness.

Funding: This work was funded by FONDECYT REGULAR 1200427 to ARF; FONIS EU-LAC T010047 to CSR, PCC, JO, JB, NLR & ARF; FONDEQUIP EQM180037 to ARF; the regional Council of the "Los Rios region" project FICR16-19, FICR19-20 and FICR21-01.

"Differences in calcium handling in Down Syndrome iPSC-derived cardiomyocytes and its influence on cell death after ischemia/reperfusion injury"

Leslye Venegas-Zamora [1,2], Wladimir Corrales [2,3], Sebastián Leiva-Navarrete [1,2]
Francisco Sigcho [1,2], Zully Pedrozo [1,4,5], Valentina Parra [1,2,5]

[1] Advanced Center of Chronic Diseases (ACCDiS), Facultad de Ciencias Químicas y Farmacéuticas y Facultad de Medicina, Universidad de Chile, Santiago, Chile.
[2] Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile.
[3] Centro de Modelamiento Molecular, Biofísica y Bioinformática (CM2B2), Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile.
[4] Programa de Fisiología y Biofísica, ICBM, Facultad de Medicina, Universidad de Chile, Santiago, Chile
[5] Network for the Study of High-lethality Cardiopulmonary Diseases (REECPAL), Universidad de Chile, Santiago, Chile

Introduction: Myocardial infarction (MI) results in irreversible heart muscle damage due to a lack of oxygen. This damage triggers cardiomyocyte necrosis induced by ischemia/reperfusion (I/R) injury, mainly due to Ca2+ overload. Unexpectedly, evidence indicates that individuals with Down Syndrome (DS) have a lower risk of coronary ischemic events and better outcomes after MI. Only ~7% of the population with DS died from MI, compared to 32% in the general population. Currently, if Ca2+ signaling is altered in DS cardiomyocytes and whether this could be a protective factor in I/R induced cell death, is unknown. Therefore, we hypothesized that: "DS cardiomyocytes show reduced cell death induced by I/R due to alterations in sarcoplasmic Ca2+ homeostasis".
Methodology: We performed a differential expression and enrichment analysis of induced pluripotent stem cells (iPSC) DS RNAseq public data. Additionally, DS iPSC were differentiated to cardiomyocytes (iPSC-CM) for 17 days, and then underwent I/R (6 and 16 h, respectively). Finally, cardiac markers and cell death were evaluated by qPCR and LDH release, respectively.
Results: Differential expression and enrichment analysis suggested that DS iPSC presents differences in Ca2+ ion homeostasis and regulation, cellular response to Ca2+and cardiac cell contraction. DS iPSC-CMs also showed decreased expression of cardiac markers and no changes in cell death after I/R compared to control iPSC-CMs.
Conclusions: Bioinformatic analyses indicate that calcium signaling is probably altered in DS iPSC, but further studies are needed for confirmation. However, there are no differences in cell death after I/R. If there is a factor present in individuals with DS that protects against ischemic events is currently under analysis.

Funding support: This work was supported by FONDECYT 1190743 (VP) and 1180613 (ZP), FONDAP 15130011 (VP, ZP), U-Redes G_2018-35 (VP, ZP) and CRP-ICGEB CHL18-04. We also thank for the ANID Doctoral scholarship to LV, WC, FS.

"Comparative analysis of the bacterial community found in soils near and far from the rhizosphere of plant growth in a copper mine tailing"

Jaime Ortega[1], Gabriel Gálvez[1], Mauricio Latorre[1,2,3]

[1] Bioengineering Laboratory; Institute of Engineering Sciences; O'Higgins University, Rancagua, Chile.
[2] Laboratory of Bioinformatics and Gene Expression, INTA, University of Chile, Santiago, Chile.
[3] Systems biology center for the study of extremophile communities from mining tailings (SYSTEMIX), O'Higgins University, Rancagua, Chile.

Copper mine tailings are an environment derived from wastes from mining activities, which present a high concentration of metals and acidic pH, which are unfavorable growth conditions for organisms. Interestingly, there are sectors in Cauquenes tailings where pines and willows can live and thrive, along with the normal microbiota of the tailings. An interesting factor that influences microbes' and plants' growth is the interaction between them and their ability to reduce environmental stress. In this work, samples from Cauquenes tailing were studied. Samples were taken from soils near willow (zone 1) and pine (zone 2) growth, and from soils belonging to Muro (zone 3) and Torre (zone 4) sectors. Results showed that the pH of zones 1 and 2 had mostly neutral pH, whereas the pH from zones 3 and 4 were mildly acidic. To research the composition of the bacterial community, DNA extraction was carried and sequenced, and OTUs were assigned. Using this information, a co-occurrence network was constructed. These results showed that in zones 1 and 2 there's a balance between positive and negative interactions between the members of the community, while in zones 3 and 4 there's mostly positive interactions. In zones 1 and 2, the most connected and abundant OTUs belonged to Proteobacteria phylum. In zones 3 and 4, the most abundant OTUs belonged to Actinobacteria phylum, while the most connected belonged to Chloroflexi and Actinobacteria phyla.

Overall, our results provide valuable information regarding the microbiome in zones with tree growth in Cauquenes tailing.

Funding: ANID FONDECYT 1190742 (ML), ANID BASAL FB210005 (ML), ANID MILENIO ICN2021_044 (ML), ANID ANILLO ACT210004 (ML) and ACT20210044 (ML), Beca ANID 21211367 (GG) and 21220593 (JO).

"Facultative endosymbionts modulate the aphid reproductive performance on wheat cultivars differing in the contents of benzoxazinoids"

Angélica González-González[1,2], Nuri Cabrera[2], **María Eugenia Rubio-Meléndez[3]**, Daniela Sepúlveda[2], Ricardo Ceballos[2], Natali Fernández[4], Christian C. Figueroa[2] and Claudio C. Ramírez[2]

[1] Doctorado en Ciencias con Mención en Biología Vegetal y Biotecnología, Instituto de Ciencias Biológicas, Universidad de Talca, Talca, Chile.
[2]Laboratorio de Interacciones Insecto-Planta, Instituto de Ciencias Biológicas, Universidad de Talca, Talca, Chile.
[3]Centro de Bioinformática y Simulación Molecular (CBSM), Facultad de Ingeniería, Universidad de Talca, Talca, Chile
[4]Instituto de Investigaciones Agropecuarias, INIA Quilamapu, Chillán, Chile.

Facultative bacteria harbored by aphids can significantly affect aphids' ecological features. However, little is known about the role of these facultative bacteria in developing aphid populations on chemically protected plants. In this work, we test the hypothesis that the endosymbiotic bacterium *Regiella insecticola* modulates the reproductive performance of the grain aphid *Sitobion avenae* in wheat cultivars that vary in their defensive compounds. For this, the variation in the intrinsic population growth rate (*rm*) of *S. avenae* genotypes, whose populations of the endosymbiotic bacterium *R. insecticola* were manipulated, was analyzed in wheat cultivars with contrasting levels of the benzoxazinoid DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzox- azin-3-one). The *S. avenae* genotypes studied were the most abundant in the asexual populations of the center-south of Chile. Our results show that in the absence of the endosymbiont *R. insecticola*, the reproductive performance of S. avenae genotypes behaves biphasic, with the lowest rm and longer time to first reproduction on wheat seedlings with medium levels of DIMBOA. However, while harboring R. insecticola, genotypes showed idiosyncratic responses. Nevertheless, a similar reproductive pattern was detected in the two most frequent genotypes harboring R. insecticola, with improved performance in aphids feeding on seedlings with intermediate levels of DIMBOA compared to aphids not harboring the endosymbiont.

The opposite trend was true at the highest concentrations of DIMBOA in all genotypes. This is the first study addressing the mediating effect of facultative endosymbionts on aphid performance in plants with different DIMBOA contents.

Funding: This work was supported by Beca Doctorado Nacional CONICYT No. 21190271I (to AG), Fondo Nacional de Desarrollo Científico y Tecnológico: Fondecyt Postdoctoral 3190544 (to MER-M), Fondecyt Regular No.1170943 (to CF), Redes-Conicyt No.170134 (to AG).

"Antarctic endophytic fungi improve the physiological and biochemical performance of strawberry plants (*Fragaria x ananassa*) against drought and high temperatures"

María Yáñez Ortega[1,2], Mario Moya[1], Sebastián Flores Valenzuela[1,2], Patricio Ramos Campos[1,3]

[1]Plant Microorganism Interaction Laboratory (PMIL), Facultad de Ciencias Agrarias y Forestales, Centro del Secano, Universidad Católica del Maule, Talca, Chile
[2]Facultad de Ciencias Agrarias y Forestales, Universidad Católica del Maule, Talca, Chile
[3]Centro de Investigación de Estudios Avanzados del Maule (CIEAM), Universidad Católica del Maule, Talca, Chile

Climate change is a problem that directly affects food security. The global warming is manifested as droughts that are detrimental to the development and yield of agricultural crops. It has been proven that, in a symbiotic association between plants and extremophile microorganisms, the latter have a fundamental role in the adaptation of plants to environmental stresses. In this study, *Fragaria x ananassa* plants were inoculated with two endophytic fungi (*Penicillium chrysogenum* and *Penicillium brevicompactum*) isolated from Antarctic plants in order to evaluate their interaction with plants against water stress. For this, different greenhouse tests were carried out under drought and high temperature conditions as a simulation of climate change conditions, where the physiological and biochemical response of inoculated and non- inoculated plants was evaluated in each treatment. It was observed that in the inoculated plants parameters such as water retention capacity and photosynthetic efficiency increased with respect to the non-inoculated ones, while proline content and lipid peroxidation decrease in inoculated plants subjected to stress due to drought and high temperatures. In addition, it was observed that the inoculation promotes a modulation of the antioxidant enzymatic activity, also of antioxidant chemical compounds and the total antioxidant capacity. Our results suggest that the functional symbiosis of Antarctic plants and microorganisms is capable of reducing the stress produced by lack of water and high temperature in crops, improving the physiological and biochemical performance of strawberry crops.

This work was supported by the project FONDECYT 1211057

"Developing a Magnetic-Tweezers for the Study of Elastic Proteins"

Tomás Hermosilla, J Andrés Rivas Pardo

Mechano-Biology Group, Microbe Genomics Lab, Centor for Genomics and Bioinformatics, Universidad Mayor, Santiago, Chile

Cell communicates through multiple stimuli, including electrical and biochemical cues. However, in the last decades, evidence has accumulated to suggest the appearance of the third kind of signal, mechanical cues, or forces that can be generated or transmitted by different molecular systems within the cell.
Dedicated instrumentation needs to be developed to study the effect of mechanical forces on biological processes, able to apply calibrated forces on protein molecules. Here, we developed high-speed Magnetic Tweezers (MT) based on a lab-made inverted microscope, which includes 3D printed components and Open-Source Design and Software. Our instrument can apply mechanical forces between 0.1 to 80 pN and distinguish protein folding transitions >100 ms, due to its 1200 Hz bandwidth.
For the calibration of our instrument, we used protein L, a bacterial protein widely used as an archetype protein for folding experiments. We measured the unfolding and refolding of protein L under force, yielding folding transitions, which we successfully adjusted using the *Freely Jointed-Chain* model. Moreover, the low mechanical drift enables us to assay the same protein molecules for hours.
Our MT instrument allows us to characterize elastic proteins' mechanical properties mimicking the cell's forces. Due to its open design, it could be easily implemented in different labs and modified accordingly. Moreover, the low mechanical drift and ability to explore the same molecule for hours turn our microscope into a good candidate for exploring drugs on mechanical protein systems, such as involved in mechanical adhesion–*epithelium infections*–or mechano-transduction–*cancer and metastasis*.

Funding: This work was supported by the National Agency for Research and Development (ANID), FONDECYT 1221064

"Biophysical characterization leucine zipper domain of the human transcription factor FoxP1"

Nicolás Paredes[1] , Jorge Babul[1], Exequiel Medina[1]

[1]Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile.

The human FoxP (FoxP) members (FoxP1-4) of the Fox family of transcription factors are master regulator proteins involved in several biologically relevant processes such as immunity and brain development. Although all Fox members share a highly conserved DNA-binding domain (FKH), FoxP proteins exclusively share a leucine zipper domain (LZ), which is defined in other models as an intrinsically disordered region able to dimerize in presence of DNA. Even when the isolated FKH in FoxP proteins is able to dimerize, the presence of the LZ domain also has a significant impact, indicating its relevance for FoxP proteins. However, whether this behavior changes due to interdomain communication or if depends on the structural and functional properties of LZ domain remains unexplored. We biophysically characterized the LZ domain to understand its structural and functional properties. For this, the structured content, dissociation properties, and stability were evaluated. Both circular dichroism and equilibrium unfolding studies indicated that LZ is mostly an unstructured protein, although this behavior changes when the protein is incubated with 2,2,2-trifluoroethanol, a chemical compound that promotes the acquisition of secondary structure, suggesting the potential ability of this protein to fold. Size exclusion chromatography indicated a Kd value of ~20 μM, 20-fold higher than the isolated FKH. Altogether, these results highlight the intrinsic disorder of the LZ domain and its low ability to dimerize, suggesting that the behavior of FoxP in the presence of LZ is a bidirectional interaction or an interdomain communication.

Funding: This work was supported by Fondo Nacional Científico y Tecnológico (FONDECYT) grant N°11200729

"Role of common catalytic residues of the vitamin kinase family in the phosphorylation of a methyl-phosphate group"

Myriam Pérez[1], Isaac Cortes[1], Nicolás Fuentes-Ugarte[1], Gabriel Vallejos-Baccelliere[1] Victoria Guixé[1], Víctor Castro-Fernández[1]

[1] Laboratorio de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Chile, Santiago, CL

In bacteria, the thiamine biosynthesis requires the phosphorylation of hydroxymethyl-pyrimidine (HMP) to hydroxymethyl-pyrimidine-diphosphate (HMP-PP), requiring the formation of hydroxymethyl-pyrimidine-phosphate (HMP-P) as intermediate. These consecutive reactions are performed by a hydroxymethyl-pyrimidine phosphate kinase encoded by the *thiD* gene (ThiD-HMPPK). The HMP-P kinase activity is unique in the ATP-dependent vitamin kinase family because consists of phosphorylation of a methyl-phosphate group (R-CH2PO4), in contrast to the classical primary alcohol group (R-OH) phosphorylation performed for other members of this kinases family, where a catalytic base (Asp or Cys) activates the alcohol and a positive charge (Arg or Lys) stabilizes the transition complex
.
A crystal structure for an ancestral ThiD-HMPPK from *Enterobacteriales* (AncEnHMPPK) in a complex with HMP-P and ATPγS showed that Lys111 could participate in the stabilization of the transition state, but Cys213 (the catalytic base) is too far from the phosphoryl acceptor group to be a catalytic residue. To determine the role of Lys111 and Cys213 activity of AncEnHMPPK, we performed site-directed mutagenesis of these residues and we characterized kinetically them. The C213A mutant was practically inactive with HMP and HMP-P substrates, while C213D enhanced HMP phosphorylation by increasing k_{cat} by 3-fold and does not significantly affect the activity with HMP-P. Finally, the K111A mutant shows that lysine residue is essential in the HMP-P phosphorylation (12-fold decrease in kcat), whereas it only decreases k_{cat} 3-fold with HMP. These results show that C213 is important for both phosphorylations (HMP and HMP-P), whereas K111 is only essencial for the HMP-P phosphorylation.

Funding: Fondecyt 1221667

"Dimer dissociation is the rate-limiting step of KaiB fold switching"

Ignacio Retamal-Farfán[1,2],Maira Rivera[1,2], Pablo Galaz-Davison[1,2], Elizabeth A. Komives[3] César A. Ramírez-Sarmiento[1,2]

[1] Institute for Biological and Medical Engineering, Schools of Engineering, Medicine and Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, Chile
[2] ANID – Millennium Science Initiative Program – Millennium Institute for Integrative Biology (iBio), Santiago, Chile
[3] Department of Chemistry and Biochemistry, University of California San Diego, La Jolla, California, USA

Cyanobacteria harbor the simplest biological clock, composed of three proteins (KaiA, KaiB, KaiC) that constitute a phosphorylation oscillator whose periodicity is regulated by the metamorphic protein KaiB. These proteins are characterized by adopting dissimilar yet thermodynamically stable structures, each with its own function. In the case of KaiB, a structural interconversion occurs between a tetramer of asymmetric dimers, or ground-state (gsKaiB), and a thioredoxin-like monomer, or fold-switch state (fsKaiB).
Although both structures and their functions are well described, how the structural interconversion occurs and what is its rate-limiting step are unknown. Here, we employ molecular dynamics simulations using structure-based models (SBM) with dual-basin Gaussian contact potentials to explore the fold-switch of KaiB. From these simulations, dimer dissociation is identified as the rate-limiting step of KaiB fold-switch, following a three-state refolding pathway with accumulation of a monomeric intermediate (gs2↔ 2gs↔2fs). Lastly, the importance of oligomer stability for the fold-switch of KaiB is demonstrated by experimental analysis of two mutants with altered periodicity, for which a shift towards population of the dimer and monomer species and local structural features compatible with fsKaiB are observed by size exclusion chromatography and hydrogen-deuterium exchange mass spectrometry, respectively.
Altogether, these results suggest that biophysical analysis of the transition state for the structural interconversion between gsKaiB and fsKaiB can be exploited for rational design of the periodicity of the cyanobacterial circadian clock.

Funding: FONDECYT 1201684, ANID Millennium Science Initiative Program ICN17_022.

"The hantavirus Gn/Gc spike stability and membrane fusion activation is modulated by multiple ionizable residues"

Eduardo A. Bignon[1,2], Esteban Rodríguez[1,2], Gianina Arata, Pablo Guardado-Calvo[3], Félix A. Rey[3], Nicole D. Tischler[1,2]

[1]Fundación Ciencia & Vida, Centro Ciencia & Vida, Laboratorio de Virología Molecular, Santiago, Chile
[2]Universidad San Sebastián, Facultad de Medicina y Ciencia, Departamento de Ciencias Básicas, Santiago, Chile
3Structural Virology Unit, Virology Department, Institut Pasteur, CNRS UMR 3569, Paris, France

Viral fusion proteins are carried on mature virions mostly in a metastable before triggering and inducing membrane fusion through their conformational change into a post-fusion form. Among class II fusion proteins, this rearrangement is induced at low pH by ionizable residues. The hantavirus Gc protein forms part of this group, and the molecular structures of the hetero-octameric Gn/Gc spikes suggest multiple key acid-sensitive residues. Based on structure-guided mutagenesis, in this work we have characterized multiple key residues in Andes virus Gn and Gc. These residues include the His285 from the ŋ1 loop from Gn, one of the three main Gn regions contacting Gc. The substitution of His285 into Tyr reduced the Gn/Gc spike stability at neutral pH. Despite its lower stability, this mutant was more resistant to acid-induced activation suggesting a role of His285 as pH-dependent switch. We also examined His665 located at the N-terminal region of Gc that in the pre-fusion form which makes hydrogen bonds with the backbone of domain III. When replacing His665 by Tyr, acidification triggered its rearrangement into Gc homotrimers albeit its fusion activity was abolished. The larger hydrodynamic radius of this trimer compared to wild type suggests its arrestment in an extended intermediate conformation. Hence, His665 seems to play a crucial role in the positioning of domain III in the pre- and post-fusion forms. Together, the results suggest a network of acid-sensitive residues necessary for both; the prefusion stability of Gn/Gc spikes and the stable low pH conformation of Gc.

Funding: ANID (Chile) grants FONDECYT 1221811 and Centro Ciencia & Vida BASAL FB210008 (Excellence Center).

"Kinetic and thermodynamic characterization of a protein cold-induced intermediate by using single-molecule force spectroscopy"

Camila Neira[1], Rodrigo Rivera[1], Andrés Bustamante[1], Oscar Aranguiz[1], María Luisa Cordero[2], Christian A.M. Wilson[1], Mauricio Baez[1]

[1]Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.
[2]Departamento de Física, Facultad de Ciencias Físicas y Matemáticas, Universidad de Chile.

The proteins undergo cold-induced unfolding as well heat-induced unfolding. This effect has been explained by the solvation of non-polar side chains exposed in the thermal denatured state originating a downward curvature of free energy dependence with the temperature. However, when the temperature is modified it is not trivial to access kinetic information and detecting the presence of kinetics intermediates from thermally denatured protein samples. Here we used optical tweezers coupled with a Laser temperature-jump device to study the mechanical unfolding of a protein between 4 and 16 °C. Both equilibrium and kinetics information can be extracted from the mechanical trajectories created by the application of a force on the protein structure. The protein, a single domain of FoxP, showed a two-state unfolding mechanism between 4 and 16 °C. The force-extension curves agree with the full unfolding of the protein since the experimental ΔLc fit with the expected value of 23 nm. The unfolding rate constants were increased with the temperature as is expected for an event driven by the thermal energy. However, when the unfolded chain is allowed to refold at lower forces, some force-extension trajectories showed a rapid fluctuation or hopping between the denatured and a compact intermediate state. Notably, the number of trajectories with hopping was more frequent at low temperatures supporting the presence of a kinetic intermediate induced by cold. In conclusion a decrease of the temperature can uncouple the cooperative refolding of a protein stabilizing intermediates states of proteins in rapid equilibrium with the native state.

Funding:: FONDECYT (number 1191153), Proyecto Enlace-Fondecyt 2022 VID, U de Chile (number ENL 10/22)

"Recombinant expression and functional characterization of the VP5 fusion protein from Halorubrum pleomorphic virus-6 in halophilic archaea"

Kevin R Chou [1], Eduardo A Bignon [1, 2], Nicole D Tischler [1, 2]

[1] Laboratorio de Virología Molecular, Centro Ciencia & Vida, Fundación Ciencia & Vida., Santiago, Chile
[2] Facultad de Medicina y Ciencia, Universidad San Sebastián., Santiago, Chile

Viruses use different strategies to enter the host cell. Enveloped viruses use a membrane fusion mechanism for cell entry. This mechanism has been widely described in eukaryotes but is still rather unknown in prokaryotes.
The *Pleolipoviridae* family includes enveloped viruses of broad morphotype diversity that infect halophilic archaea. Recently we have demonstrated that the infection of cells by Halorubrum pleomorphic virus-6 (HRPV-6) involves a virus-cell membrane fusion process, which is induced by the viral spike protein (VP5) and triggered by an S-layer component from the host *Halorubrum sp. SS7-4*. Yet, the molecular details of this mechanism remain unknown.
To study the fusion mechanism of VP5, the aim of this work consisted in establishing a heterologous expression system in native conditions. In order to achieve this, we cloned the transcriptional unit of VP5 into an expression vector for haloarchaea and we tested the transformant colonies by PCR and western blot. A higher expression signal of VP5 was observed in the host cells *Hrr. sp. SS7-4* than in *Haloferax volcanii*. Using a R18 fluorophore dequenching assay, the fusion activity of the recombinant VP5 protein was tested by cell-cell fusion assays. A higher level of lipid mixing was observed in *H. volcanii* than with *Hrr. sp. SS7-4*, particularly at 55 °C.
Our results show that our recombinant system of the viral fusion protein VP5 shows higher expression in the natural host but conserves a greater functionality in *H. volcanii*.

Funding: Volkswagen Foundation (Germany) and ANID (Chile) grants FONDECYT 1221811 and Centro Ciencia & Vida BASAL FB210008 (Excellence Center).

"Combining optogenetics and CRISPR-Cas technologies for GPD1 gene repression in yeast"

Camila Baeza[1,2], David Figueroa[1,2], Diego Ruiz[1,2], Vicente Rojas[2,3], Luis F. Larrondo[2,3], Francisco Salinas[1,2]

[1]Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile.
[2]ANID–Millennium Science Initiative–Millennium Institute for Integrative Biology (iBIO), Santiago, Chile.
[3]Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile.

The alcoholic fermentation or 'glucose fermentation pathway', it is a metabolic process carried out by yeast, by which glucose is converted into ethanol, carbon dioxide and other by-products such as acetate and glycerol. The main determinants of glycerol production are *GPD1* and *GPD2* genes, which encodes for glycerol-3-phosphate dehydrogenase isoenzymes. The industrial importance of the glucose fermentation pathway has prompted important efforts toward the transcriptional control of genes responsible of its products yield. In this work, we combined optogenetics and CRISPR-Cas technologies to achieve the transcriptional repression of the *GPD1* gene. We used a variant of the FUN-LOV optogenetic switch known as FUN-LOV^{SP} (single plasmid) for light-activated expression of the catalytically inactive version of the Cas9 protein (dCas9), which was fused to three different endogenous transcriptional repressor domains (RD) from yeast proteins (Tup1, Mig1, and Ume6). Thus, we generated a genetic circuit in yeast that combines optogenetic and CRISPRi (interference) technologies. By using different guide RNAs (gRNAs), we directed the dCas9-RD to the *GPD1* promoter, assessing its transcriptional repression through the luciferase reporter gene. The results showed that under constant blue light, the transcriptional activity of the *GPD1* promoter was repressed in 31,3% by dCas9-Mig1(RD), 47,6% using dCas9-Tup1(RD), and 74% by dCas9-Ume6(RD). Therefore, the combination of the FUN-LOV^{SP} switch with the CRISPRi system allowed the transcriptional repression of *GPD1*, potentially permitting the redirection of the metabolic flux in the glucose fermentation pathway.

This work was supported by ANID-FONDECYT 1210955

" Unleashing the full power of homebrew reagents for low-cost RT-LAMP"

Javiera Aviles[1,2], Tamara Matute[2], Isaac Nuñez[2], Maira Rivera[2], Javiera Reyes[2]
César A. Ramírez-Sarmiento[1,2], Fernán 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, Santiago, Chile

The COVID-19 pandemic evidenced the challenges in countries of the global south to efficiently respond to the urgent demand of molecular tools to combat health crisis under the current scenario, in which their production is mostly centralized in the global north. Moreover, several molecular biology techniques for detection of pathogens, such as LAMP, RT-LAMP, and RPA, have been developed to overcome the disadvantages associated with reaction time, reagent cost and access to specific equipment related to the use of RT-PCR.
Paradoxically, many of the enzymes involved in these techniques are now in the public domain, allowing freedom of operation. However, the plasmids typically used for protein expression in bacteria are products protected by nondistribution agreements.
In this work, the present the design of open plasmids for IPTG-based induction of protein overexpression in *E. coli* cells. We validated the use of these plasmids for efficient protein overexpression using a free-use GFP (fuGFP). Then, we successfully overexpressed and purified public domain DNA polymerases and reverse transcriptases, namely BstLF, MMLV, and HIV1-RT, which we employed to generate a fully open RT-LAMP molecular kit. Our results exemplify how to unleash the full power of open-source tools, from DNA to enzyme production to testing, for the greater benefit of all.
These molecular tools, designed in collaboration with FreeGenes, OpenBioeconomy Lab and Ginkgo Bioworks, are part of the ReClone collection, whose components are compatible with common standards for DNA assembly (uLoop/MoClo/Golden Gate) and can be distributed to anyone under a new legal tool known as openMTA.

Funding: ANID-CONCYTEC covbio0012, ANID Millennium Science Initiative Program ICN17_022

SPONSORS


TALCA
UNIVERSIDAD
CHILE


Universidad
Andrés Bello®


Universidad
de Concepción


ACCDiS
ADVANCED CENTER FOR CHRONIC DISEASES


GERO


iBio
Instituto Milenio de
Biología Integrativa


TCL
Group


GENEXPRESS
Life Science Business


FERMELO
BIOTEC


ThermoFisher
SCIENTIFIC


MERISTEM


GRUPO
SIBI