Sociedad de Bioquímica y Biología Molecular de Chile

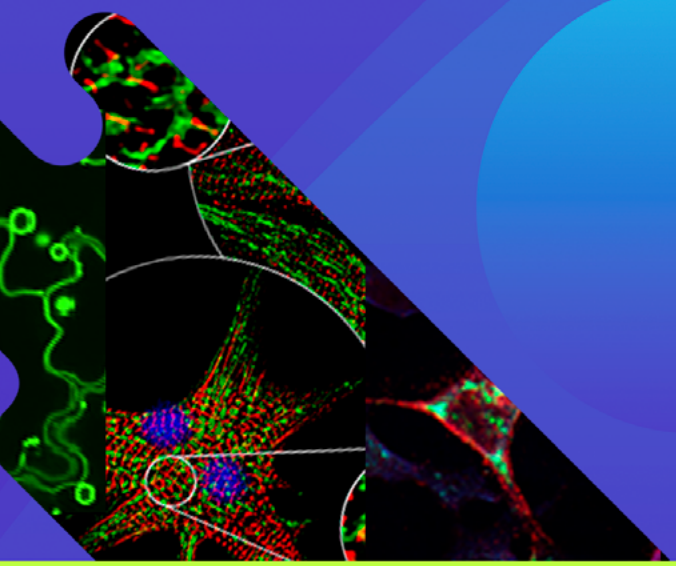
CHILEAN SOCIETY OF PLANT BIOLOGISTS

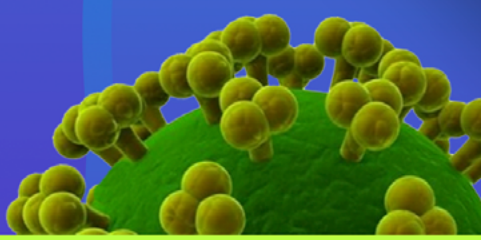
XLII annual meeting of the Chilean Biochemistry and Molecular Biology Society (SBBMCh)

XIV annual meeting of Chilean Society of Plant Biologists (CSPB)

"A MOLECULAR AND INTEGRATIVE VIEW OF BIOLOGICAL SYSTEMS"

October 8 to 11, 2019. Hotel Gavina Sens, Iquique





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Opening Lecture

The Sweet Heart: Mechanism of Cardioprotection by a GLP-1 Receptor Agonist

Gottlieb Roberta A [1], Andres Allen Mariano[2], Chengqun Huang[3], Song Yang Kristina[4].
(1) Medicine, Professor, Cedars-Sinai Medical Center, Los Angeles, US
(2) Medicine, Assistant Professor, Cedars-Sinai Medical Center, Los Angeles, US
(3) Medicine, Project Scientist, Cedars-Sinai Medical Center, Los Angeles, US
(4) Medicine, Research Associate, Cedars-Sinai Medical Center, Los Angeles, US

Recent clinical trials have reported that antidiabetic drugs--notably glucagon like peptide 1 receptor agonists (GLP1Ra) and sodium glucose cotransporter 2 inhibitors (SGLT2i)--reduce the incidence of adverse outcomes in patients with type 2 diabetes mellitus (T2DM). We hypothesized that these drugs confer cardiac benefit through effects on mitochondrial turnover in the setting of post-infarction remodeling. We evaluated the efficacy of a short-acting glucagon-like peptide-1 receptor agonist (GLP1Ra)—specifically 2-quinoxalinamine, 6,7-dichloro-N-(1,1-dimethylethyl)-3-(methylsulfonyl)-, 6,7-dichloro-2-methylsulfonyl-3-N-tert-butylaminoquinoxaline (DMB) – in attenuating adverse LV remodeling. We also examined the role, if any, of mitochondrial turnover in this process. Wild-type, Parkin knockout and MitoTimer expressing mice were subjected to permanent coronary artery ligation, then treated briefly with DMB. LV remodeling and cardiac function were assessed by histology and echocardiography. Autophagy, mitophagy and expression of metabolic enzymes involved in fuel utilization were also examined. Mitochondrial biogenesis was inferred from MitoTimer protein fluorescence. We found that DMB given post-infarction significantly reduced adverse LV remodeling and the decline of cardiac function. This paralleled an increase in autophagy, mitophagy and mitochondrial biogenesis. Interestingly, DMB treatment was effective in non-obese mice as well as mice with diet-induced obesity and even in mice made insulin deficient with streptozotocin injection. However, the salutary effects of the drug were lost in Parkin knockout mice, implicating Parkin-mediated mitophagy as part of its mechanism of action. Our findings suggest that enhancing Parkin-associated mitophagy after infarction is a viable target for therapeutic mitigation of adverse remodeling.

National Institutes of Health P01 HL112730 (PI Roberta Gottlieb); R01 HL132075 (co-PIs Roberta Gottlieb and Jennifer Van Eyk); and R01 HL144509 (co-PIs Van Eyk and Gottlieb)

Osvaldo Cori Lecture

Rhapsody of proteins: structure and function
Rapsodia de proteínas: estructura y funcion

J Babul[1].
(1) Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile

El estudio de las relaciones entre la estructura y función de las proteínas continúa siendo uno de los principales desafíos de la biología estructural. Las características particulares de la estructura de las proteínas les permiten desempeñar un rol crucial en la integración de los procesos biológicos por medio de la regulación de sus funciones, la que surge de los cambios estructurales que éstas experimentan provocados por la unión de ligandos o por la unión a otras proteínas. Estos cambios pueden modificar la estabilidad, la flexibilidad conformacional, la comunicación alostérica entre sitios de unión y el estado de agregación de subunidades. La comprensión de estos cambios implica el explorar los principios básicos que están detrás de las relaciones entre estructura y función.

Se presentará una historia del estudio de esas relaciones a través de varias proteínas, la que comprende: los principios del plegamiento de las proteínas usando citocromo *c* como modelo; la importancia fisiológica de las propiedades alostéricas y el mecanismo de regulación de la fosfofructoquinasa, su vía de desplegamiento y el papel que cumplen las interacciones entre subunidades en su estabilidad y actividad; el efecto del sustrato en la estabilidad mecánica de la glucoquinasa a nivel de molécula individual y el proceso de dimerización por la vía de intercambio de segmentos del factor de transcripción FoxP, donde regiones desordenadas y de mayor flexibilidad afectan la dinámica y termodinámica del proceso.

Por cierto que la rapsodia continúa.

(Fondecyt 1170701)

PABMB Lecture

It´s a matter of time: circadian clock and defense responses crosstalk in *Arabidopsis thaliana*

Marcelo Yanovsky[1].
(1) Fundacion Instituto Leloir, Buenos Aires, Argentina

Abstract

In an ever-changing world, possessing the ability to adapt to, and even predict, daily and seasonal environmental changes, represents a valuable feature for living organisms. Indeed, in most organisms, multiple physiological and developmental processes are driven by an internal timekeeping mechanism known as the circadian clock, which accurately fine tunes the timing of these processes contributing to an enhanced fitness. In plants, the endogenous biological clock regulates multiple processes, and in turn, several clock regulated signaling pathways feedback to control clock function. Given that plants do not have any specialized immune cells, each individual cell must regulate and balance the high energy consuming stress responses with other cellular functions, such as growth. The circadian clock has been shown to modulate plant immunity, and the role of several clock genes in the control of biotic stress responses has been addressed, but whether plant-pathogen interactions modulate clock function is still unclear. Recently, we found that an enhanced disease susceptibility (*eds*) mutant displayed alterations in circadian rhythms and clock associated responses. Also, by implementing a mapping by sequencing approach we were able to determine the identity of the mutation responsible for the *eds* phenotype, which had remained unknown for more than 20 years and the identity of this gene, with its implications for the operation of the circadian network, will be discussed. Simultaneously, we found that an infection with Pseudomonas syringae strongly alters the expression of most core clock genes, as early as 1h post-infection in wild type (wt) plants and that this effect was attenuated in the *eds* mutant. Furthermore, we identified new clock mutants that turned out to be more susceptible to *Pseudomonas syringae* infection. Thus, these results represent a novel example of the relevance of correct timing of defense responses and reinforce the idea of strong crosstalk between biotic stress stimulus and the *Arabidopsis* circadian clock.

Severo Ochoa Lecture

Unravelling the role of stress kinases in obesity-related diseases

Guadalupe Sabio[1].
(1) Myocardial Pathophysiology, Centro nacional de Investigaciones Cardiovasculares

Protein kinases are the key components of almost every signalling pathway involved in normal development and disease. MAPKs play a key role in the regulation of diverse cellular programs and participate extensively in the control of cell fate decisions such as proliferation, differentiation, and death, as well as in the regulation of stress responses. The main stress-activated MAPKs are the p38 and c-Jun N-terminal kinase (JNK) families. The p38MAPK family has four isoforms encoded by distinct genes located tandemly in 2 chromosomes: p38a, b, g and d. Although the role of all p38s have been thought to be similar, we think the less-studied members p38g and d have different and specific regulation and function. We will discuss about our recent findings that suggest that these kinases have specific function and regulation. We will focus our attention in their implication in obesity related diseases.

Symposium - Proteomics and its role on the study of virulence and host-pathogens interactions

Schistosome and its host interactions as revealed by proteomics: from biology to vaccine design

William Castro-Borges[1].
(1) Ciências Biológicas, Instituto de Ciências Exatas e Biológicas, Universidade Federal de Ouro Preto

For the last two decades proteomic investigations have been applied to understanding the biology of *Schistosoma mansoni*, a blood-dwelling helminth parasite of humans. In this conference it will be presented the major approaches devoted to the proteomic characterization of the parasite´s tegument and alimentary tract, the two major host-parasite interfaces. The conference will focus on the range of biological processes that have been uncovered and how the accumulated findings could be translated into novel avenues for therapeutics, diagnosis and vaccine design.
CAPES, CNPq, Newton Fund and UFOP

Dynamic imaging of host-pathogen interaction in surrogate host models

Francisco Chávez[1].
(1) Departamento de Biología, Facultad de Ciencias, Universidad de Chile

Microbial infections are characterized by a constant interplay between pathogen and host with pathogens exploiting an array of host cell functions during infection and their hosts reacting with appropriate defense response. To understand this complex interaction, scientist have been turning their efforts using *in vivo* infection models. The use of mammalian models to identify and understand the virulence factors of human pathogens is essential. However, to overcome the limitations associated with using mammalian models of infection for visualizing the dynamic of host-pathogen interaction, researchers have turned to analyzing this interplay in surrogate hosts. Important features of host-pathogen interactions have been discovered using non-mammalian hosts. Therefore, model hosts such as the nematode *Caenorhabditis elegans*, the social amoeba *Dictyostelium discoideum* and the zebrafish *Danio rerio* have been increasingly used for *in vivo* host–pathogen interaction studies. In adittion, live-cell imaging studies on these model hosts benefit from genetic and genomic tools that have been generated over the years in these models. A major challenge for studying intracellular bacterial pathogens is to understand the molecular bases of disease development during host infection. Knowledge about virulence factors abundance is therefore crucial to gain a quantitative view of the pathogenic functions. Although numerous proteomic studies of *in vitro*-grown microbial pathogens are well known for many pathogens, *in vivo* host-pathogen metaproteomic approaches are still unavailable. Here we used a combination of global proteomic profiling with live cell imaging for deciphering host–pathogen interactions for *S.* Typhimurium and *P. salmonis.* By approaching the *in vivo* dynamic of host-pathogen interaction with a systems biology approach we discover novel virulence factor and host defense mechanism in these important intracellular pathogens.

Fodecyt 1120209

Dynamic rewiring of the human interactome by interferon signalling

Leonard Foster[1].
(1) Biochemistry & Molecular Biology, Medicine, University of British Columbia

The type I interferon (IFN) response protects cells against viral pathogens by inducing the transcription of hundreds of IFN-stimulated genes (ISGs). Transcriptomic and biochemical approaches have established comprehensive catalogues of ISGs across species and cell types, but their antiviral mechanisms remain incompletely characterized. Here, we apply a combination of quantitative proteomic approaches to map IFN-induced rearrangements in the human protein-protein interaction network. Differential network analysis reveals interaction rewiring across a surprisingly broad spectrum of cellular pathways. We identify IFN-dependent interactions mediating novel regulatory mechanisms at the transcriptional and translational levels, including a specialized ribosome that buffers ISG synthesis. Our map of the IFN interactome provides a global view of the complex cellular networks activated during the antiviral response, placing ISGs in a functional context, and serves as a framework to understand how these networks are dysregulated in autoimmune or inflammatory disea**se.**

Canadian Institutes of Health Research

The virulence of *Trypanosoma cruzi*: A proteomic perspective

Jorge González[1].
(1) Tecnología Médica, Ciencias de la Salud, Universidad de Antofagasta

Chagas disease is caused by *Trypanosoma cruzi*, a protozoan that affects 8 million people worldwide. The parasite invade the host, resist its immune response and proliferate. In all of these process many different molecules may act in a sequential and concerted manner. In fact, many of them have already described in the literatura as *T.cruzi* virulence factors. However, there is still a lot to learn about what virulence really means in *T. cruzi* and which parasite molecules are absolutely required or critical to make *T. cruzi* one of the most successful pathogens to invade, survive and persist in a mammalian host. Here, using Ultra High Perfomance Liquid Chromatography coupled to mass spectrometry, we compared the proteome of two *T.cruzi* trypomastigote cell lines (CL) derived from a single *T. cruzi* clone. For 30 years one of them, named C8C3***hvir*****,** was passaged in mice, where it maintained its high virulence. The other, called C8C3***lvir***, was maintained in axenic culture, where its virulence was attenuated. Then, protein extracts of these two CL were digested with trypsin and aliquots were analyzed by LC-MS/MS. The analysis, using MaxQuant, led to identification proteins, of which several of them displayed differential expression in C8C3***hvir*** versus C8C3***lvir***. In conclusion, C8C3***hvir*** expresses many proteins at higher levels with respect to the C8C3***lvir***. The potential role of these protein on parasite virulence will be discussed.

Semillero Grant SEM 17-2 and Bridge Grant, University of Antofagasta.

Symposium - Plant Breeding

Genome wide association analysis (gwas) of grapevine quality traits for breeding new seedless varieties

Paola Barba[1], Inti Pedroso[2], Miguel García[1].
(1) Instituto de Investigaciones Agropecuarias
(2) Universidad del Desarrollo

Although Chile leads global table grape exportations by volume variety renewal just started, providing an opportunity for the development of new genotypes, locally adapted, with international quality standards. To accelerate its development, our breeding program implemented marker assisted selection (MAS) for seedlessness, screening over 8.000 progenies each season. We also use a high-throughput classic forward genetics approach to study complementary quality traits.

First, we used reference-based genotyping-by-sequencing of table grape collection at INIA germplasm repository and more than 500 accessions from seven families of the breeding program. SNPs were filtered and imputed, resulting in 30.000 SNPs with 2% missing data.

Multiple Component Analysis allowed to understand the genetic diversity of our breeding material. Genetic distances among individuals was used to search for family outliers and self-pollinated progenies. Population STRUCTURE analysis fitted a model with nine populations, where seven of them represented breeding families.
GWAS was performed for eight quality traits, using Linear Mixed Models with kinship relatedness correction. Additive heritability estimates ranged from 0.5, for quantitative traits with high environmental effects such as rachis or cluster weight, up to 0.9 for the mayor locus controlling seedlessness. High heritable traits such as seed dry weight, berry size and berry shape were significantly associated to one, three and four loci, respectively.

As in seed dry weight case, traits with high heritability and few loci are prone for MAS. Development of high-throughput rhAmpSeq tests for routine screening of loci associated with berry shape and size could facilitate its use in breeding programs.

GWAS for the genetic improvement of growth and wood traits in *Eucalyptus* and *Populus* species

Fernando Guerra[1], Paulina Ballesta[1], Freddy Mora[1], David Neale[2], Haktan Suren[3], Jason Holliday[3], Nicolle Serra[4], Rodrigo Hasbún[5], Randi Famula[2], Jim Richards[2], Brian Stanton[6], Oliver Fiehn[2], Mark Davis[7].
(1) Instituto de Ciencias Biológicas, Universidad de Talca, Chile
(2) Department of Plant Sciences, University of California Davis, USA
(3) Department of Forest Resources and Environmental Conservation, VirginiaTech, USA
(4) Semillas Imperial SpA, Chile
(5) Facultad de Ciencias Forestales, Universidad de Concepción, Chile
(6) Greenwood Resources, USA
(7) National Renewable Energy Laboratory, USA

Eucalyptus globulus and *Populus trichocarpa* are important species for producing biomass suitable for wood products, pulp and paper or bioethanol, among others. Genomic-based breeding approaches have a significant potential in the context of the genetic improvement of productive traits. In that framework, this work summarizes the results coming from two studies based on the analysis of the relationship between SNP-markers and a suite of complex traits involved in biomass production and chemical composition of wood. First, we performed a GWAS, utilizing 1,7M SNPs, to identify significant associations determining phenotypic variation in a *P. trichocarpa* clonal trial established in the United States, genotyped by exome-capture. Second, we utilized SNP-markers to assess the ability of seven genomic selection models to predict growth traits in a *E. globulus* progeny trial, established in Chile, and genotyped with a 60k-SNP chip. GWAS on *P. trichocarpa,* by single-SNP and sliding-windows tests identified a set of significantly associated SNP-markers, which were distributed across the exome and related to genes encoding proteins belonging to different functional classes. Analyses in *E. globulus* indicated that a variant of the method "Ridge Regression-BLUP", with previous selection of predictor variables (RRBLUP-B), and the model "supervised principal component regression (PCR)" presented the greatest predictive ability.Results contribute to a comprehensive characterization of the genetic mechanisms controlling complex productive traits in fast growing tree species. At the same time, provide new understanding of how genome-wide data could be applied to support the develop of novel breeding strategies for tree genetic improvement.

Acknowledgments: We would like to thank FONDECYT (Grant no.1170695), CONICYT-PCHA Program (Grant no. 21160624) and Advanced Hardwood Biofuels Northwest Project (USDA-AFRI Program Grant no. 2011-68005-30407).

Strong gluten strength of modern durum wheat (*Triticum turgidum* L. var *durum*) genotypes associate with higher levels of alanine than weak lines

Jorge Araya-Flores, Parada Roberto, Adriano Costa De Camargo, Iván Matus[1], **Schwember Andrés R [2]**.
(1) Sede Quilamapu, Instituto de Investigaciones Agropecuarias, Chillán, CL
(2) Ciencias Vegetales, Agronomía e Ingeniería Forestal, Pontificia Universidad Católica De Chile, Santiago, CL

Durum wheat (*Triticum turgidum* L. var. *durum*) grain quality is an important trait that encompasses nutritional value, protein(gluten) content and quality and yellowness. In this study, twelve modern CIMMYT (International Maize and Wheat Improvement Center) genotypes were analyzed and classified as weak, medium or strong gluten strength. SDS-PAGE gel electrophoresis analysis of allelic variation for high (HMWGs) and low molecular weight glutenin subunits (LMWGs) were carried out, and RT-qPCR expression of glutenin-related genes was performed along grain filling. In parallel, the determination of the aminoacidic composition during grain development was studied through HPLC. Overall, strong gluten strength lines showed higher expression levels of LMW glutenin-related genes between 21-35 days post anthesis (DPA), and they exhibited up to 43.5% more alanine than the weak lines at 42 DPA, which coincided with previous higher expression levels during seed development of putative alanine amino transferase genes of the strong genotypes. These results contribute to improve durum wheat grain quality that is of high value for the pasta industry and the consumers.

CONICYT Fondecyt Regular 1161298

Unlocking and assessing the performance of local genetic resources as strategy to mitigate climate challenges

A. Zurita-Silva[1], C. Alfaro[1], D. Castillo[1], V. Arancibia[1], M.a. Montoya[1], P. León[1], V. Arenas-Morales[2], G. Nuñez-Lillo[2], C. Meneses[2].
(1) Instituto de Investigaciones Agropecuarias (INIA), Centro de Investigación Intihuasi, Colina San Joaquin s/n, La Serena, Coquimbo, Chile
(2) Universidad Andrés Bello, Facultad Ciencias de la Vida, Centro de Biotecnología Vegetal, Santiago, Chile, República 217, Santiago, Chile

Introduction.
Food production is facing water deficit problems related to climate change, new extremes in heat and precipitation regimes and drought events. Global freshwater shortage is one of current biggest challenges, often associated to misuse and increased consumption demands paralleled with desertification process. This requires novel resilient food sources.

Methodology.
By usinga combined strategy to characterize at both morpho-physiological, functional, and molecular level to identify both genetic and phenotypic diversity and tolerance to abiotic stresses, a panel of 96 Chilean genotypes of *Chenopodium quinoa* from INIA Quinoa Breeding Program were genotyped (24 SSR microsatellites) and phenotyped, including proximal analysis. This included functional approaches at field, and proximal determinations to understand responses to stress.

Results and Conclusions.
Genetic and phenotypic characterization of 96 selected lines is discussed, determining the population structure, key phenotypical traits, and assessed effects of water deficit to determine tolerance and selection of genotypes after drought treatment. Expression patterns for canonical drought are also discussed, and advantages of combined approaches for breeding purposes.

Acknowledgements.
FIC Atacama, and MINAGRI PMG Quinoa–INIA.

Symposium - Glucose and vitamin C transporters in health and disease. A tribute to Juan Carlos Vera

Happy 20th anniversary to a vitamin C transporter: from the biochemistry to the biomedicine.

Maite A. Castro. Instituto de Bioquímica y Microbiología, Facultad de Ciencias, UACh Center for Interdisciplinary Studies on Nervous System (CISNe), UACh Janelia Research Campus, Howard Hughes Medical Institute HHMI. macastro@uach.cl; castrom@janelia.hhmi.org

Maite A. Castro[1].
(1) Instituto de Bioquimica y Microbiologia, Ciencias, Universidad Austral de Chile

The reduced form of vitamin C -ascorbic acid- is an important enzyme cofactor in the biosynthesis of collagen, carnitine and catecholamines. It is a powerful antioxidant highly concentrated in adrenal gland, pituitary, liver, spleen and brain. Ascorbic acid uptake by cells occurs via a specific transport system, stereospecific and driven by a sodium gradient: sodium vitamin C transporters, SVCTs. In brain, SVCT2 is exclusively expressed in neurons, in hypothalamus glial cells and in choroid plexus epithelial cells. Thus, ascorbic acid is also an important neuroprotective and neuromodulator agent. During the last 19 years we have been making steady progress in the mechanisms of communication between neurons and glial cells and the use of ascorbic acid as inter cellular messenger. Here, we will describe the regulation of neuronal ascorbic acid transporters under synaptic activity and how it is affected in aging and neurodegenerative diseases.

Fondecyt 11070065, 1110571, 1151206, 1191620, Anillo-CONICYT ACT1401.

From Glucose and Vitamin C transporters to Glycogen metabolism in the seminiferous tubule

Concha Ilona I [1], Slebe Juan C[1].
(1) Bioquímica y Microbiología, Ciencias, Universidad Austral de Chile, Valdivia, CL

Spermatogenesis is a complex physiological process that involves cell proliferation, meiotic division and final cell differentiation of post-meiotic cells into spermatozoa. During this process male germ cells also undergo a process of metabolic differentiation, in which spermatids respond differentially from spermatocytes in the expression and function of glucose transporters (GLUTs) and in the metabolism of glucose and glycogen. In spermatocytes glucose and dihydroascorbic acid uptake is performed through GLUT1, in the case of spermatids and spermatozoa, the uptake of these substrates is also carried out through GLUT3. Glycogen is considered as the main source of glucose, however in male germ cells this polymer can play another role, which has not been totally elucidated. In all these processes the link of germ cells with Sertoli cells is of great relevance. Sertoli cells express functional GLUTs1-4 transporters and the muscle isoform of the glycogen synthase (MGS) is phosphorylated and inactive. Positive regulation of MGS can be achieved through a greater availability of glucose-6P or via Wnt/b-catenin signaling, in addition to other factors. The differences found in germ cells in conjunction with Sertoli cells could be explained by the need for a specific metabolic process to support cell differentiation or, in some cases, cell viability.

FONDECYT 1110508 (IC) and 1141033 (JCS)

Hypothalamic glucosensing and food intake regulation

Barahona Maria J, Magdiel Salgado , Estefania Tarifeño-Saldivia, Roberto Elizondo-Vega, **Maria De Los Angeles García-Robles**[1].
(1) Biología Celular, Ciencias Biológicas, Universidad De Concepción, Concepción, CL

Introduction:
Glucose is a crucial modulator of feeding behavior. By acting in peripheral tissues and the CNS, it controls the secretion of hormones and neuropeptides and modulates the activity of ANS. GLUT2 is required for several glucoregulatory responses in the brain, including feeding behavior, and is localized in the hypothalamus and brainstem, which are the main centers that control this behavior. In the hypothalamus, GLUT2 and glucokinase (GK) have been detected in glial cells, known as tanycytes, which line the basal walls of the third ventricle (3V).

Methods:
We used 3V injections of an adenovirus encoding a shRNA against GLUT2 or GK and the reporter EGFP. Efficient *in vivo* knockdown in rat hypothalamic tissue was demonstrated by qPCR and Western blot analyses. The specificity of cell transduction in the hypothalamus and brainstem was evaluated by confocal microscopy.

Results:
The altered mRNA levels of both orexigenic and anorexigenic neuropeptides showed a loss of response to increased glucose in the 3V. Feeding behavior analysis in the fasting-feeding transition revealed that GLUT2/GK-knockdown rats had increased food intake and body weight, suggesting an inhibitory effect on satiety.

Discussion:
The suppression of GLUT2/GK expression in tanycytes leads to the loss of the neuron-responses to intracerebroventricular increased glucose, with consequences in the feeding behavior, which supports the participation of the tanycytes in an indirect mechanism of glucose detection in the hypothalamus.

Fondecyt 1180871

The good, the bad and the ugly of intracellular vitamin C transport

Coralia I. Rivas[1], Roa Francisco J[1], Peña Eduardo[1], Carola Muñoz-Montesino[1].
(1) Fisiopatología, Ciencias Biológicas, Universidad De Concepción

Vitamin C, also known as ascorbic acid, is a broad-spectrum antioxidant that is essential for the survival of humans, whom must obtain it from dietary sources since they cannot synthesize it. There are two biologically active forms of vitamin C, the oxidized form, dehydroascorbic acid and the reduced form, ascorbic acid. Vitamin C plays most of its biological functions intracellularly and is acquired by cells with the participation of specific membrane transporters. Most cells express two different transport systems for vitamin C, a specific transport system for dehydroascorbic acid, and a specific transport system for ascorbic acid. Dehydroascorbic acid is transported through the GLUT family of facilitative glucose transporters, and ascorbic acid is transported by the SVCT family of sodium-coupled transporters. Even when it is known that vitamin C is necessary for different functions in all animals and plants, and in human physiology, its participation in human pathologies, especially in diseases such as cancer, is still a controversial and a complex issue. The lack of information on the mechanisms used by cancer cells to acquire vitamin C has complicated understanding the role of this vitamin in cancer. Recently, we have analyzed in vitro the ability of cancer cells to acquire, metabolize and compartmentalize vitamin C and the possible effects it could have on cancer. Coralia I. Rivas: corivas@udec.cl

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Symposium - Plant Genomics

Genomic and metagenomic exploration of microbial endophytes and population structure of *Araucaria araucana* in Chile

Jaime Alarcón[1,2], Sebastián Márquez[1,2], Claudio Meneses[1,2], Eduardo Castro-Nallar[1,2].
(1) Universidad Andrés Bello, Center for Bioinformatics and Integrative Biology (CBIB)
(2) Universidad Andrés Bello, Centro de Biotecnología Vegetal

Araucaria araucana (Araucaria; Class = Pinopsida; Family = Araucariaceae) is an endangered conifer with a fragmented and relict distribution in southern Chile and Argentina. Logging (banned in 1990), wildfires, overgrazing, invasive trees, and extensive human harvesting of Araucaria seeds have historically threatened Araucaria populations. More recently, the Chilean forest authority reported extensive damage, which is characterized by the browning of branches and needles following a "bottom-up" pattern. While 90% of *A. araucana* population is affected, there is only a 2% mortality rate in Chile (CONAF). The disease was dubbed DFA as "foliar damage of the Araucaria tree" for its acronym in Spanish.

While there are several hypotheses regarding the cause of DFA including approximately a 10-year drought in the region, the widespread nature of the disease which covers all of its geographic distribution at various intensities, plus gardens, nurseries, and public squares, suggests the influence of a pathogen, opportunistic or otherwise. Here, we use amplicon sequencing targeting the 16S rRNA and ITS taxonomic marker genes to reveal the structure and composition of Araucaria´s microbial communities throughout its geographic distribution (n > 600). Community analyses suggest that Araucaria´s microbial communities are structured primarily within the tree by tissue, and secondarily by sampling site, i.e., the Andes or Nahuelbuta mountain ranges and north/south gradient. We complement these analyses with shotgun metagenomic sequencing to look into potential pathogens outside the purview of amplicon sequencing, as well as RAD-Seq analysis to understand Araucaria´s population structure.

Funded by CONAF (Corporación Nacional Forestal; Chilean National Forestry Corporation)

Identification of Quantitative Trait Genes using global gene expression analysis and association analysis for berry textural properties in grapevine

Identification of Quantitative Trait Genes combining global gene expression analysis and association analysis for berry SIZE and textural properties in grapevine

Nallatt Ocarez[1], Reynaldo Núñez[1], Nicolás Jiménez[1], Jorge Lagrèze[1], Vicente Salas[1], Diego Osorio[1], Bruno Defilippi[1], **Nilo Mejía[1]**.
(1) Breeding and Biotechnology Unit, La Platina Regional Research Center, Instituto de Investigaciones Agropecuarias.

In table grape, besides seedlessness, berry size and berry firmness are perhaps among the most interesting traits at consumer level. Seedlessness is fairly understood at genetic and molecular level, however berry size and firmness have been weakly characterized. These quality-related traits are among the key attributes that can benefit from molecular diagnosis for the selection of targeted phenotypes within breeding programs, making the development of new varieties a more efficient process.

The aim of this study was to characterize the genetic architecture of the most important berry quality attributes like berry size and firmness. Based on a mapping bi-parental population (n > 500) derived from the cross of Muscat of Alexandria x Crimson Seedless that was characterized at genetic level with more than 5,000 SNP-based markers and at phenotypic level for seedlessness, berry size and textural properties for up to four seasons we performed QTL analysis.

Results reveal the simple genetic architecture of seedlessness, and the complex and very complex nature for berry size and textural properties respectively. We describe correlations at genetic level among these traits that explain the difficulty to develop a perfect new variety. For firmness that is decomposed into several textural properties and several tens of QTLs we applied a transcriptome differential expression analysis to reduce the apparent complexity of this trait.

At a stage were soft and firm berries differentiate, QTL analysis coupled with transcriptomic enrichment analyses based on Gene Ontologies shows that the biological processes that are significantly overrepresented in soft berries are related to auxins: efflux, homeostasis, regulation of metabolic and catabolic process, response, polar and intracellular transport; as well as pectin catabolic process, secondary cell wall biogenesis, cell wall modification, organization and biogenesis and flavonoid biosynthetic process. This knowledge is of great value for the establishment of predictive methodologies for breeding and agronomic management practices based in growth regulators.

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Elucidating the clonal diversity and genetic variation in Vitis vinifera cv. Cabernet Sauvignon

Claudio Urra[1,3], Catalina Pavez-Reyes[1,3], Andrea Minio[4], Matthieu Miossec[2], Cristóbal Balladares[1], Alvaro Castro[3], Dario Cantu[4], **Claudio Meneses[1]**.
(1) Centro de Biotecnología Vegetal, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago de Chile, CL
(2) Center for Bioinformatics and Integrative Biology, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago de Chile, CL
(3) UC Davis-Chile, Life Sciences Innovation Center, CL
(4) Department of Viticulture and Enology, University of California Davis, Davis, California, US

Cabernet Sauvignon (CS) is the most iconic variety for the Chilean viticulture industry. Numerous selections (named as clones by viticulture industry) of CS are available, which show significant differences in agronomic and enological traits. These differences have been originated from the accumulation of somatic mutations during thousands of asexual propagation cycles. However, the genetic variation that underlies these differences remains mostly unknown. The goul of this study was to characterize the genetic variation and clonal diversity in *Vitis vinifera* cv. Cabernet Sauvignon (CS). Thus, we re-sequenced eight CS clones (plus three biological replicates) using the Illumina HiSeq2500 sequencer, and we obtained a mean coverage of 30X. We mapped an average 98% of the reads and detected by GATK an average of 3,581,252 variants (83.4% SNP and 16.6% InDel) among CS clones compared to the reference genome. The frequency and distribution of the variants were homogeneous detecting 5.3 SNP/Kbp and 1.1 InDel/Kbp for all analyzed clones. Considering the global nucleotide diversity among the clones, three of them showed significant differences while five seem the same genome. We found an average of 1,395 unique variants from CS clones with an average of 1.66% located in coding regions. We were able to validate the most promising clone-specific variants by amplicon sequencing. Besides, we have used the custom bioinformatic pipeline to detect clone-specific markers for 'Sauvignon blanc', 'Chardonnay', 'Merlot' and 'Pinot noir' varieties. The results will be used to develop a high-throughput genotyping platform for clonal identification.

This work was supported by FONDECYT 1160584 and CORFO 13CEI2-21852.

Plataforma Genómica para el estudio de especies locales de interés agronómico: Maqui (*Aristotelia chilensis* Mol.) y Pepino Dulce (*Solanum muricatum* Aiton)

Francisco Correa[1], Francisca Pozo[1], Francisca Beltran[1], Jorge Perez-Diaz[1], Ricardo Verdugo[2], Adriana Bastias[3], **Sagredo Boris Boris [1]**.
(1) Lab Genomica y Biotecnología, INIA-Rayentue, Rengo, CL
(2) Universidad de Chile, CL
(3) Universidad Autónoma de Chile, CL

El Maqui (*Aristotelia chilensis* Mol.) y Pepino Dulce (*Solanum muricatum* Aiton.) son especies de gran interés agronómico principalmente por sus frutos especiales que se caracterizan por su alto contenido en compuestos beneficiosos para la salud. En maqui es una especie silvestre que se distribuye entre las regiones de Coquimbo y Aysen, y el caso del pepino dulce, es una planta introducida por los Incas que se cultiva principalmente en las regiones de Coquimbo y Valparaíso. En ambos casos se están realizando esfuerzos por desarrollar variedades que sean más fáciles de cultivar, altamente productivas, con frutos atractivos para los consumidores con cualidades de post-cosecha permitan una adecuada distribución en los mercados nacionales e internacionales. Con el propósito de generar una plataforma genómica que apoye estos esfuerzos de mejoramiento genético y además facilitar conservación de sus poblaciones naturales hemos secuenciado (illumina NexSeq 550), ensamblado (*de novo*) y obtenido borradores de sus genomas, con 326 Mb (140X) y 918 Mb (56X) para Maquí y Pepino dulce, respectivamente. La calidad de los ensamblajes fueron evaluados por alineamiento de secuencias de sus transcriptomas (RNAseq), disponibles en base de datos y a través de la herramienta BUSCO. Estos borradores nos permitió identificar secuencias microsatélites (SSR) en los respectivos genomas, algunos de los cuales se han validado y utilizado en la caracterización de poblaciones y colecciones de ambas especies.

Fondecyt 1161377; Fondecyt 11150551; FONDEQUIP EQM140157 and U-Redes: \"U-Genoma" (VID, U. de Chile)

Symposium - Simposio Cono Sur: Aptamers as molecular tools for biomedical applications

Development of aptamers for therapeutic purposes: a success story

Victor Manuel Gonzalez[1].
(1) Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Hospital Ramón y Cajal, Madrid, España

Aptamers are single stranded nucleic acids (ssDNA or RNA), isolated from oligonucleotide libraries by in vitro selection, by a known SELEX method. These molecules adopt three-dimensional structures that allow them to interact with their targets. Since Toll-like receptor 4 (TLR4) mediates brain damage after stroke, development of TLR4 antagonists is a promising therapeutic strategy for this disease. Our aim was to generate TLR4-blocking DNA aptamers to be used for stroke treatment. From a random oligonucleotide pool, we identified two aptamers with high affinity for human TLR4 by systematic evolution of ligands by exponential enrichment (SELEX). Optimized truncated forms (ApTLR#1RT, ApTLR#4FT) were obtained. Our data demonstrate specific binding of both aptamers to human TLR4 as well as a TLR4 antagonistic effect. ApTLR#4F and ApTLR#4FT showed a long-lasting protective effect against brain injury induced by middle cerebral artery occlusion (MCAO), an effect that was absent in TLR4-deficient mice. Similar effects were obtained in other MCAO models, including in rat. The absence of major toxicology aspects and the good safety profile of the aptamer further encourage its future clinical positioning for stroke therapy and possibly other diseases in which TLR4 plays a deleterious role.

Molecular imaging with aptamers

Victoria Calzada[1].
(1) Centro de Investigaciones Nucleares, Universidad de la República, Montevideo, Uruguay

Aptamers are the perfect tools to recognize molecular targets and can be easily modified to confer additional functions. Their versatile characteristics make aptamers ideal for diagnostic applications. Furthermore, the small size and polyanionic nature of aptamers may lead good tissue uptake, and may minimize the residence time in the liver and kidneys, providing potentially useful features for in vivo approaches. Thus, aptamers as targeting components are biomolecules with strong potential for the development of molecular imaging probes. Molecular imaging involves in vivo characterization and measurement of biologic processes at the molecular level. These can reveal complex structures and dynamic interactive processes located deep in the sample that are otherwise difficult to decipher. For successful imaging, localization of the target, acquisition time, signal component, energy and detection equipment, are features to be considered to the design of the methodology. Several strategies have been employed aptamers in the design of molecular imaging probes and have already been included into a variety of molecular imaging modalities. However, the future potential of aptamers in the fields of imaging research and diagnostics is still a challenge.

Omics as a source for novel biomarkers and aptamer development

Pohl Milón[1].
(1) Centro de Investigación e Innovación, Universidad Peruana de Ciencias Aplicadas-UPC, Lima, Peru

Pathogenic microorganisms are a common problem to human health, agriculture, and alimentary industries, among others, causing deaths and economic loses worldwide. The prompt detection of infectious microorganisms can save lives or allow tailored interventions to reduce financial impacts. Here, we use large genomic, transcriptomic, and proteomic data sets to identify novel biomarkers that are abundant, species-specific, and absent in the host. Particular focus will be given to recent applications of next-generation sequencing (NGS) for assessing the cellular abundance of potentially novel biomarkers, called ribosome profiling (RP). On the other hand, the use of NGS for the identification and evolution of aptamers during SELEX (Systematic evolution of ligands by exponential enrichment) will be discussed. Altogether, a series of aptamer interactions with biomarkers of P. falciparum, T. cruzi, E. coli, and M. tuberculosis will be presented.

Subtractive combinatorial libraries for differential markers in complex mixtures by one-step selection of aptamers

Martín Radrizzani[1].
(1) Centro de Estudios de Salud y Medio Ambiente, Universidad de San Martín-CONICET, Buenos Aires, Argentina

Most neurodegenerative diseases have no cure and their diagnosis are performed once clinical symptoms appear. In Parkinson´s disease, the pathognomonic sign of the disease is the appearance of Lewis bodies that are the product of alpha-synuclein aggregates. In order to apply a gene therapy to the affected dopaminergic neurons, we decided to direct nanoparticles to these neurons by using aptamers. We developed a system for selecting aptamers in a single step which are endocytosed by the SH-5YSY dopaminergic neuronal cell line. We demonstrated that the selected aptamers are endocytosed by the cells. When these aptamers are bound to the albumin nanoparticles, this capacity is improved at concentrations between 1 to 10 pM. The aptamers retain their ability to detect in a murine model. We used the albumin nanoparticles with quantum dots in mouse histological sections in which receptors were observed. Finally, we observed the targeting of nanoparticles in tissue cultures in less than three hours. Therefore, we demonstrated that the aptamers selected allows to direct nanoparticles to neurons to be further endocytosed. More studies are needed to demonstrate the specificity of the dopaminergic neuronal targeting in vivo.

Symposium - Oxidative stress, ROS and related pathologies

Past, present and future of antioxidants in the context of cancer

Pedro Miguel Buc[1].
(1) Quimica y Farmacia, Ciencias de la Salud, Universidad Arturo Prat

It is now widely accepted that reactive oxygen species (ROS), act as signaling molecules involved in the regulation of a wide variety of biological processes. Nevertheless, the initial focus was focused on deleterious and detrimental effects caused by free radicals on macromolecules and tissues. For instance, lipid peroxidation was considered one of the most important cytotoxic mechanisms leading to cell injury under condition of oxidative stress. Nowadays, emerging evidence suggests that ROS played essential roles in physiologically relevant signaling as well as aberrant signal transduction. Regarding this latter condition, alterations in redox signaling are involved in several human diseases: i.e. cancer cells have increased ROS levels in comparison to their normal counterparts. In this context, such redox dysregulation in cancer cells may provide a way to kill cancer cells via elevating ROS to highly toxic levels intracellularly. On the other hand, several authors still suggest that antioxidants, by equilibrating redox homeostasis, should be employed against cancer cells. Interesting, in the last years such controversy has been progressively sorting out in favor of a pro-oxidant rationale approach. This lecture is a brief historical review about uses and misuses of both oxidants and antioxidants. Other related-issues will be further discussed in this Symposium. The main topics will be about mechanisms of ROS regulation and redox homeostasis. A potential link between redox dysregulation and the implication of such oxidative stress in human diseases will be presented for discussion. (*This work was supported by Fondecyt regular 1190577)*

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Flavonoids in human health: relevance and bioactivity as regulators of redox signaling

Fraga Cesar G. [1,2,3].
(1) Fisicoquimica, Farmacia y Bioquimica, University of Buenos Aires, Buenos Aires, AR
(2) Instituto de Bioquímica y Medicina Molecular (IBIMOL), Universidad de Buenos Aires-CONICET, Buenos Aires, AR
(3) Department of Nutrition, University of California, Davis, CA 95616, US

It is increasingly accepted that in mammals, the bioactivities of flavonoids (as parent compounds or as metabolites) can be mostly ascribed to their chemical and physical interactions with proteins and lipids. As a paradigm of flavonoids, we studied (-)-epicatechin (EC) as a molecule providing benefits in hypertension, insulin-resistance, and models renal and intestinal dysfunction, studied in experimental rodents. In these pathological conditions, we characterized EC and EC-related compounds (ECrc)) by their capacity to modulate cell redox signaling through extra- and intracellular actions, and by redox and non-redox changes. Therefore, interacting from the outer layer of cell membranes, ECrc can alter lipid rafts, receptors and functional proteins/enzymes (e.g. NADPH oxidases). These interactions would be relevant for cells present in certain tissues that are exposed to ECrc, e.g., the intestine and the vascular system. Once incorporated into cells, ECrc can interact with: i) proteins and enzymes that define oxidant levels, e.g. enzymes that generate and/or metabolize superoxide, H2O2 and NO; ii) redox sensitive transcription factors; and iii) oxidant species. Meanwhile, i) and ii) can be relevant in any tissue exposed to ECrc, the scavenging of oxidants will be relevant only in the upper digestive tract, in which the concentration of ECrc is high especially after the consumption of an EC-rich food.It can be concluded that the understanding of the molecular mechanisms involved in the biological actions of flavonoids is necessary to define recommendations in terms of which plants can better promote health, and/or the amounts necessary to provide health effects.

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HO-1, a Host Antioxidant Protein With Antiviral Activity Against Herpes Simplex Viruses

Pablo Alberto Gonzalez[1].
(1) Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile

Herpes simplex viruses (HSVs) type 1 (HSV-1) and type 2 (HSV-2) are highly prevalent in the human population. These viruses establish lifelong infections in the host and sporadically reactivate either, symptomatically or asymptomatically. The most common clinical manifestations produced by HSVs are skin lesions, yet these viruses can also elicit more severe diseases, such as blindness and life-threatening encephalitis. Importantly, HSV infect numerous cell types including epithelial cells, neurons and immune cells, among others. While neurons are infected to establish persistent infection, immune cells are likely infected to negatively modulate the host antiviral response to the virus. Although antivirals exist against HSVs, these are somewhat ineffective for alleviating the most frequent HSV-related symptoms. Heme oxygenase-1 (HO-1) is a host enzyme that plays antioxidant roles in the cell and can be pharmacologically induced. HO-1 transforms free heme into three cytoprotective products, namely biliverdin, free iron and carbon monoxide, the latter which can act as an intracellular signaling molecule. Recent studies within our laboratory show that HO-1 induction in epithelial cells, neurons and immune cells has antiviral effects against HSVs and that some of the antiviral effects elicited by HO-1 induction are recapitulated by CO. Furthermore, inducing HO-1 *in vivo* reduces HSV-related disease, suggesting that this host antioxidant factor may eventually be modulated to help treat HSV infection.

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Superoxide radicals at the macrophage phagosome: contribution of oxidative stress to pathogen killing

Lucia Piacenza[1].
(1) Bioquímica, Facultad de Medicina, Universidad de la República

Macrophages are the first line defense of the innate immune response towards pathogens. Membrane-bound NADPH-oxidase (NOX-2) is a pivotal player in this defense mechanism. Early activation of NOX-2 is mediated by pathogen engulfment in the phagocytic vacuole. The activation gives rise to superoxide radical (O2·-) production that can last 30-90 minutes after pathogen internalization. The toxicity of O2·-at the phagosome has been largely claimed but has not been well established. Its anionic nature, limits its diffusion across membranes restricting its toxicity at the site of its production. Nevertheless, at the acidic macrophage phagosome (pH≤5), O2·- can be found in its protonated form, perhydroxyl radical (HO2·, pKa=4.8), a neutral species and a more potent oxidant than O2·-. Using *T. cruzi*as a model, the toxicity of O2·-/HO2·radicals were evaluated at the macrophage phagosome. Results reveal that O2·-diffuses across anion channels whereas HO2·freely permeates parasite membranes. Once in the parasite cytosol (pH*ca.*6,5-7), HO2·deprotonates yielding intracellular O2·-. O2·-can lead to oxidative damage to iron-sulfur-containing enzymes while HO2·can initiate lipid-peroxidation. Importantly, in the presence of nitric oxide (·NO) generated in immunostimulated macrophages by the inducible nitric-oxide-synthase, intracellular peroxynitrite formation was detected and enhanced O2·-/HO2·toxicity towards the internalized parasite. The above data was confirmed using *T. cruzi*overexpressing cytosolic Fe-superoxide dismutase being more resistant to macrophage killing confirming both, a direct and indirect (·NO-dependent) toxicity caused by O2·-/HO2·in the acidic macrophage phagosome.

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Symposium - Simposio Chile – SBBq Brasil: Outreach, scientific education and the social role of science

The teaching of the general philosophy of science as a measure of protection for the professional scientific practice

Carlo Apablaza[1].
(1) Departamento de Filosofía, Facultad de Humanidades, Universidad de Santiago de Chile

The question about whether the teaching of philosophy has any utility is long-standing in Chilean society. Nevertheless, philosophers often fail to establish what social objectives the teaching of this discipline would meet. In my talk I intend to justify that the teaching of the *general philosophy of science* can be considered as a support tool for a specific purpose, namely, the increase in *citizen scientific literacy*. Currently, this kind of literacy is an objective that OECD links to the progress and welfare of the countries and I will show how this is related to the general philosophy of science and to the professional scientific practice as follows: First, I will explain why such literacy is important showing its social implications. I will also explain, analyzing the administration of Donald Trump, how the detriment of it directly affects professional scientific research. Then, I will talk about the *attitudes towards science* and explain how they are a factor that is positively correlated with scientific literacy. Then, I'll examine cases of current scientific disinformers and show how their actions generate hostile attitudes towards science in our citizens. Finally, I will show how the transfer of scientific content or data does not change these attitudes of our citizens and that the most effective way to achieve this objective is to instruct them in a more complete picture of science. In other words, I'll conclude that the widespread teaching of the philosophy of science improves citizen scientific literacy and therefore protects professional scientific research.

Vaccines, public health and social media

María Paz Bertoglia[1].
(1) Escuela Salud Pública, Facultad de Medicina, Universidad de Chile

Following a downward trend, in recent years, cases of vaccine-preventable diseases have increased. The world is once again experiencing major measles outbreaks, with expected health, social and economic consequences. Causation is multifactorial, nevertheless there's no doubt that one of the causes is the decrease in immunization coverage due to increased vaccine hesitancy. This has become such a serious problem, that the World Health Organization has identified vaccine hesitancy as one of the 10 greatest threats to public health in 2019. At the center of this phenomenon is the loss of confidence in vaccines and the baseless fears promoted by misinformation and Fake News in social and mass media. But social media have also demonstrated the ability to be a great ally in dealing with misinformation and vaccinate hesitancy. Each social media platform has its own communication codes, target audiences, and treatment of specific issues related to vaccines and other subject that may affect the public health. Successful outreach strategies have been developed, such as an official Facebook site, succeeded in doubling HPV vaccine coverage in Denmark. Emphasis should be given on one of the best practices in scientific communication: do not promote false equivalences when there is scientific consensus (vaccines, climate change, among others). In this case, the role of communicating should be based on providing information in appropriate formats for decision-making, not on promoting artificial debates that gives equal validity to both positions, a situation that may to confuse the population.

Low-cost equipment for Biochemistry Lab classes: Do it yourself or ask your students to do it!

Eduardo Galembeck[1].
(1) Biochemistry and Tissue Biology, Biology Institute, University of Campinas

In this talk, I will present the results obtained from an online automatic titrator and a low-cost spectrophotometer designed, developed and used to support biochemistry lab classes. The automated online titrator is composed of two scales on which stock solutions of titrants are deposited. The titrant solutions are added to the titrating using peristaltic pumps. A magnetic stirrer on which a Becker beaker is deposited contains the titrating solution, having its base illuminated for better visualization of the titration reactions. A pH meter is immersed in the titrant. All measurements of the sensors (scale and pH) are broadcasted online to a web page. The peristaltic pumps and magnetic stirrer controllers are also made online through the same page.

The low-cost, and portable spectrophotometer was constructed by using an RGB LED and a light detector. The RGB LED can be set to deliver visible light at various wavelengths. We used the low-cost spectrophotometer for protein dosage by the Bradford method, and for glucose dosage by the Somogyi-Nelson method. The results obtained showed a correlation higher than 0.98 with the same measurements made with a commercial spectrophotometer.

The use of Arduino technology has enormous potential to increase biochemistry laboratories. The online automatic titrator allows the realization of titrations in a faster way and with automatic registration of the data. It allows students to devote more time to the planning of the experiments and interpretation of the results. The spectrophotometer presented results equivalent to commercial equipment.

Biochemistry and Art in undergraduate teaching

Silvia Prado[1].
(1) Biochemistry and Molecular Biological, Federal University of Parana

The project Biochemistry and Art is intended to provide a collaborative learning method among undergraduate medical students. In this project, students are asked to write a poem or short story about a specific topic of Biochemistry. This strategy leads to a new way of thinking about Biochemistry and to the development of new skills.

Symposium - Plant Metabolism

Papaya sugarcane: using endogenous molecular and biochemical mechanisms to “soften” biomass for bioethanol production

Marcos Buckeridge[1].
(1) Laboratory of Plant Physiological Ecology, Department of Botany, Institute of Biosciences; Systems and Synthetic Biology Center (S2B), INOVA-USP; National Institute of Science and Technology of Bioethanol (INCT-Bioethanol); University of São Paulo, São

Besides being a source of sugar, sugarcane is one of the primary sources of ethanol. The expansion of sugarcane for biofuel production is possible without effects on food production or biodiversity. To achieve this goal, we need to use not only the free sugars but also the cell wall (CW) carbohydrates. A large number of hydrolases from microorganisms are now available. Combined into enzyme cocktails, they are used in industry but still represent an expensive step in the process. Another limitation is the process of biomass pretreatment, which is used to make CWs available to hydrolysis. To bypass these barriers, we have been trying to find ways to endogenously “soften” the CWs. After the determination of the chemical composition of sugarcane CWs, we found that the development of the gas spaces in sugarcane roots (aerenchyma) includes an endogenous CW modification system that controls wall degradation. We think that it may be possible to “install” parts of this system in the whole plant to ease pretreatment and gain control on hydrolysis and described the CW modifications that lead to aerenchyma. We assembled a collection of CW-related genes of sugarcane belonging to the classes of transcription factors, miRNAs, and hydrolases. Together, these mechanisms became part of a strategy to turn sugarcane CWs more amenable to hydrolysis. We are now using integrative biology to reengineer a sugarcane variety capable of softening its own CWs in a similar fashion as fruits such as papaya do during ripening. We nicknamed it “papaya sugarcane project.” https://www.botany.one/2019/07/how-to-break-down-cell-walls-in-sugarcane/

Plant Metabolic Plasticity: Genetic bases and Environmental interactions

Fernando Carrari[1].
(1) Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE-UBA-CONICET) Ciudad Universitaria, C1428EHA Buenos Aires, Argentina. Universidad de Buenos Aires. Facultad de Agronomía. Cátedra de Genética. Buenos Aires, Argentina.

Genetic bases of metabolic traits have been studies in several plant species. However, their understanding is restricted to few molecular mechanisms which cannot explain the wide phenotypic plasticity linked to these kinds of traits, especially when genotypes are exposed to extreme environments. Our research group is focused on (epi) genetic factors determining biochemical processes in harvestable organs of main *Solanaceae* species (tomato, potato, tobacco). In this talk I present and discuss results coined from i) analyses of the epigenetic nature of metabolic content regulation in the fruit of tomato, particularly in the vitamin E biosynthesis, ii) how metabolism shifts in response to changes in temperature and iii) analyses of perturbations on gene expression linked to genomic stress associated to hybridization between divergent genotypes and their relations with hybrid vigor (heterosis) in higher plants.

Increased protein lipoylation alters tomato development

Michael Handford[1], María Paz Covarrubias[1], Karina Olivos[1], Pamela Cabedo[1].
(1) Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, CL

Lipoic acid (LA) is a functional metabolite with powerful antioxidant capacities present in eukaryotic and prokaryotic organisms. However, LA is also the prosthetic group of several key multi-subunit enzyme complexes, including pyruvate dehydrogenase and α-ketoglutarate dehydrogenase of the TCA cycle. LA biosynthesis and incorporation into these target proteins (lipoylation) proceeds *de novo* or via the salvage pathway. During the former, octanoyl transferase (LIP2) uses octanoyl groups linked to an acyl carrier protein to transoctanylate target proteins. Subsequently, lipoyl synthase (LIP1) catalyses the final step by inserting two sulphur atoms into the prosthetic group. Whilst a number of the enzymes have been functionally-characterised in *Arabidopsis*, our aim is to identify and evaluate the role of this pathway in a fruit-bearing species. To do so, we identified two proteins in tomato (*Solanum lycopersicum*) with the molecular characteristics of LIP1. We call these proteins SlLIP1 and SlLIP1p, with 78% and 84% amino acid identity with AtLIP1 and AtLIP1p, respectively. Confirming bioinformatic predictions, SlLIP1 is mitochondrial whereas SlLIP1p is plastidial, as shown by confocal microscopy after transient leaf transformation with YFP fusion proteins. Moreover, both proteins rescue carbon source requirements and lipoylation levels of an *Escherichia coli* lipoyl synthase mutant (*lipA*), acting as lipoyl synthases in this heterologous system. Furthermore, stable over-expression of SlLIP in tomato produces transcriptional alterations in genes encoding proteins involved in LA metabolism. The raised lipoylation of target proteins in the TCA cycle correlates with altered fruit development in several over expressing lines.

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The exciting road to clarify the biosynthesis of aroma compounds in Chilean fruit

Moya-Leon Maria A [1], Raul Herrera[1].
(1) Instituto de Ciencias Biológicas, Universidad de Talca, Talca, CL

Aroma is an important attribute of fruit quality. The aroma is due to a complex mixture of volatile compounds, and although many compounds are recognized as aroma-active, esters with fruity notes are crucial. As a way to clarify the biosynthesis of esters, the Chilean papaya fruit (*Vasconcellea pubescens*) has been used based in its outstanding fragrant and pleasant aroma. The esters produced by the fruit were quantified by headspace-SPME technique. The most important ones in terms of abundance and sensory impact were ethyl acetate, ethyl butanoate, methyl butanoate and butyl acetate. Also these esters increment their production during ripening. Alcohol acyltransferases (AAT) can synthesize esters from alcohols and acyl-CoAs as subtrates. The gene VpAAT1 was isolated and characterized, and then expressed in yeasts providing a functional enzyme. The biochemical characterization of the enzyme and the model of its 3D structure, in addition to several bioinformatic analyses, provided a comprehensible picture of the contribution of VpAAT1 in the synthesis of esters by the fruit. Other aromatic fruit species have also been studied: *Fragaria chiloensis* and *Physalis peruviana*. The aroma of each of them is determined by a different mixture of several esters, and particular AATs are in charge of their synthesis. The complete scientific road to explain aroma biosynthesis in fruit species will be reviewed.

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Symposium - Computational Physical Chemistry and Biochemistry at the Multiscale Level: Different Points of View for Solving the Same Problem

Multi scale QM-MM modeling of protein reactivity and spectroscopy

Dario Ariel Estrin[1].
(1) Química Inorgánica, Analítica y Química Física, Ciencias Exactas y Naturales, Universidad de Buenos Aires

Computational techniques for modeling biomolecules have emerged during the last decades as an important tool to complement experimental information, providing atomic resolution insight into the dynamics, chemical reactivity, and spectroscopic properties of enzymes. An elegant way to explore chemical reactivity and spectroscopy in proteins consists in employing multi level quantum classical schemes (QM-MM). We will present in this talk an overview of our group QM-MM implementation, as well as an application to the representative example of the molecular basis of peroxiredoxin action. This extremelly relevant protein family detoxifies peroxides by a very efficient thiol oxidation reaction. We will also show results for the prediction of vibrational and electronic spectra of heme proteins by performing QM-MM molecular dynamics simulations and real time DFT calculations.

Universidad de Buenos Aires, CONICET, ANPCYT

Revealing the Origin of Enzyme Catalysis by Merging Theory and Experiments: Towards the Design of New High Value Molecules

Sergio Martí[1], Raquel Castillo[1], Katarzyna Swiderek[1], Maite Roca[1], Kemel Arafet[1], Daria De Raffele[1], Natalia Serrano[1], Miquel A. Galmes[1], **Vicent Moliner[1]**.
(1) Química Física i Analítica, Universitat Jaume I

Computational Chemistry techniques based on the combination of Quantum Chemistry and classical Molecular Mechanics (QM/MM) have been extensively applied to the study of enzyme catalysis. Merging these techniques with experimental methods has allowed to acquire a deep knowledge of the reaction mechanisms of these complex but highly efficient biocatalysts at the molecular level. We will focus in this communication on aspects such as the controversial debate on whether protein dynamics are linked to the chemical reaction step,[1] the role of the quantum tunelling and the electrostatic effects contributions to catalysis,[2] or the relevance of compression effects in enzymatic methyl transfer reactions.[3-5] Recent results obtained in our laboratory in these different lines of research will be summarized in this communication **References** [1] Luk, L.Y.P. et al. Proc. Nat. Acad. Sci. USA. 2013, 110, 16344−16349. [2] Krzemińska, A.; Moliner, V.; Świderek, K. J. Am. Chem. Soc. 2016, 138, 16283−16298. [3] Świderek, K.; Tuñón, I.; Williams, I.H.; Moliner, V. J. Am. Chem. Soc. 2018, 140, 4327–4334. [4] (a) Świderek, K.; Tuñón, I.; Moliner, V.; Bertran, J. Arch. Biochem. Biophys. 2015, 582, 68–79. (b) Nödling, A.R. et al. Angew. Chem. Int. Ed. 2018, 57, 12478−12482. [5] (a) Kholodar, S. A.; Ghosha, A. K.; Świderek, K.; Moliner, V.; Kohen, A. Proc. Natl. Acad. Sci. USA. 2018, 115, 10311−10314. (b) Ren, R.; et al. Nature Commun. 2018, 9, Article number: 3243. (c) Serrano-Aparicio, N.; Świderek, K.; Moliner, V. Eur. J. Med. Chem. 2019, 164, 399−407.

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Two better than one. Catalysis in the dimeric Dihydrofolate Reductase from *Thermotoga Maritima*

Inaki Tunon[1], Ruiz-Pernía Jose J.[1], Vicente Moliner[2].
(1) Química Física, Químicas, Universidad de Valencia, Valencia, ES
(2) Química Física, Universitat Jaume I, Castellón, CL

Dihydrofolate Reductase from *Thermotoga maritima* (TmDFHFR) is a dimeric thermophilic enzyme that catalyzes the hydride transfer from the cofactor NADPH to dihydrofolate less efficiently than other DHFR enzymes, such as the mesophilic analogue *Escherichia coli* DHFR (EcDHFR). Using QM/MM potentials we show that the reduced catalytic efficiency of TmDHFR is not due to its dimeric nature but most likely due to differences in the amino acid sequence that stabilize the M20 loop in an open conformation, which prevents the formation of some interactions in the transition state and increases the number of water molecules in the active site. However, dimerization provides two advantages to the thermophilic enzyme; it protects its structure against denaturation by reducing thermal fluctuations and it provides a less negative activation entropy, toning down the increase of the activation free energy with temperature. Our molecular picture is confirmed by the analysis of the temperature dependence of enzyme kinetic isotope effects in different DHFR enzymes.

Ministerio de Ciencia, Innovación y Universidades (Spain). Project PGC2018-094852-B-C22

String method: revealing mechanisms of complex enzymatic reactions

Kirill Zinovjev[1], Iñaki Tuñón[2].
(1) School of Biochemistry, University of Bristol
(2) Departamento de Química Física, Universidad de Valencia, Burjassot, ES

One of the challenges in theoretical characterization of biochemical processes is the flexibility of an enzyme-substrate complex at physiological temperature, which results in a huge number of conformations that must be visited by a computer simulation. A common approach to tackle this problem is to determine an adequate reaction coordinate that brings the system from reactants to products, thus allowing to efficiently distribute the computational load along the reaction path. However, this approach becomes problematic when the reaction mechanism is not known or multiple mechanisms are possible, which is often the case for enzymatic reactions. In recent years we have developed a methodological framework to study complex processes in condensed phases, including enzymatic reactions. Our approach is based on the string method – a technique for tracing minimum energy paths on multidimensional free energy surfaces. The method allows to rapidly obtain both the reaction mechanism and the activation free energy of a given process with minimal user intervention. We briefly present the method and show multiple successful applications to complex enzymatic processes that would have been challenging to study using standard free energy calculation techniques.

Ministerio de Economía y Competitividad, Spain (project CTQ2015-66223-C2-2-P)

Symposium - Plant Biotic Interactions

Interaction between the model non-legume plant *Arabidopsis thaliana* and the symbiotic nitrogen-fixing rhizobia *Ensifer meliloti* RMP110 for improved plant growth and nitrogen nutrition

Grace Armijo[1], Tatiana Kraiser[1], María Paz Medina[1], Javier Santiago[1], Isabel Pochet[1], Ana Zúñiga[2], Bernardo González[2], Gutiérrez Rodrigo A[1].
(1) Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile, Santiago, CL
(2) Laboratorio de Bioingeniería, Facultad de Ingeniería y Ciencias, Universidad Adolfo Ibáñez, Santiago, CL

Nitrogen (N) is an essential macronutrient whose availability in the soil has a critical role in plant growth and development in natural as well as in agricultural environments. Plants acquire N directly from the soil and in some cases can be provided by interacting with N-fixing bacteria. These type of interactions are well described in legumes, but are also observed in some non-legume species, that are unable to form nodules.

Our goal was to evaluate whether a functional association between a non-legume model plant, *Arabidopsis thaliana*, and N-fixing model bacteria can occur. Through genetic and biochemical evidence we found that *Ensifer meliloti* enhanced *A. thaliana* growth under N-deficiency conditions. We showed that this growth promotion is mediated by bacterial N-fixation and allows the plant to complete the life cycle under severe N-deficiency. We visualized bacterial root colonization through different types of microscopy, locating this bacterium in the rhizosphere associated to the root epidermis. Finally, we demonstrated that *A. thaliana* homologs of key regulatory genes involved in legume-rhizobium interactions are required for growth promotion mediated by *E. meliloti*.

Our results indicate a non-canonical interaction between *A. thaliana* and *E. meliloti* for plant nutrition under N-deficiency with conserved molecular mechanisms of legume-rhizobium interactions for improved growth under N-limiting conditions. Understanding these plant-bacteria interaction mechanisms could have important agronomic implications, reducing the use of N-fertilizers in non-legume crops for a more sustainable agriculture.

Fondo Desarrollo Áreas Prioritarias (FONDAP), Center for Genome Regulation (15090007), Instituto Milenio iBio – Iniciativa Científica Milenio MINECON, Fondo Nacional Desarrollo Científico y Tecnológico (FONDECYT) 1180759

Pectin Methylesterases Modulate Plant Homogalacturonan Status in Defenses against the Aphid *Myzus persicae*

Francisca Blanco-Herrera[1,2], Christian Silva-Sanzana[1].
(1) Centro de Biotecnología Vegetal, Facultad de Ciencias de la Vida, Universidad Andrés Bello
(2) Millennium Institute for Integrative Biology, CL

Aphids are phloem feeder insects distributed worldwide and transmit approximately 50% of the known viral diseases to crops. The pectic polymer homogalacturonan (HG) is a pivotal element for plants immunity against pathogens, however, its role during aphid infestation remains unexplored. Considering this, we evaluated the dynamics of HG and its modifying enzymes on the early stage of plant-aphid interaction. To this, we used Arabidopsis plants and a *Myzus persicae* clone possessing PME activity on its salivary secretions as a model of interaction. Additionally, the influence of pectin methylesterase (PME) activity on aphid settling and feeding behavior was evaluated by free choice assays and Electrical Penetration Graphs (EPGs), respectively. Our results revealed that HG status and HG-modifying enzymes are significantly altered during the early stage of the plant-aphid interaction. Aphid infestation induced a significant increase in total PME activity, methanol emissions, PL activity, and abundance of de-methylesterified HG. Conversely, inhibition of PME activity led to a significant decrease in the settling and feeding preference of aphids. Furthermore, we demonstrate that the PME inhibitor AtPMEI13 has a defensive role during aphid infestation since *pmei13* mutants were significantly more attractive for aphid settling and the phloem ingestions performed by the insects were longer on these mutants comparing to the wild type genotypes.

Fondo Nacional de Desarrollo Científico y Tecnológico (1170259), and Iniciativa Científica Milenio (Instituto Milenio iBio).

Host-manipulation or mutualism: *Paraburkholderia phytofirmans* PsJN and the molecular networks underlying its effects in *Arabidopsis thaliana*

Poupin María J. [1,3], Daniela Orellana[1,3], Tania Timmermann[1], Andrea Vega[2], Bernardo González[1,3], Thomas Ledger[1,3].
(1) Laboratorio de Bioingeniería, Facultad de Ingeniería y Ciencias, Universidad Adolfo Ibáñez, Santiago, CL
(2) Millennium Institute for Integrative Biology (iBio), CL
(3) Center of Applied Ecology and Sustainability (CAPES), CL

The key role-played by microbiomes in the life histories of eukaryotic organisms is becoming increasingly evident. Nevertheless, the complexity of the ecological interactions taking place in nature (microbe-microbe-host-environment) hampers the possibility to get a comprehensive understanding of the molecular mechanisms governing these interactions, and also to realize the evolutionary implications for each of the interacting organisms. Although reductionist, the study of binary host-microorganism interactions becomes a reliable tool to unravel the roles that microbes have in adaptive and evolutionary processes of macroorganisms. *Paraburkholderia phytofirmans* PsJN is a well-known beneficial bacterium, which interacts with different plant species both in their rhizospheres (soil surrounded and influenced by the roots) and internal tissues. We have found that this bacterium induces profound changes in the development of *Arabidopsis thaliana*, accelerating its life cycle, flowering and senescence; and inducing adaptation to biotic (i.e. Induced stress resistance to *Pseudomonas syringae* DC3000) and abiotic (i.e. Induced stress tolerance to salinity) stresses. Here, we will discuss about the molecular mechanisms and gene networks that underlie these effects. On the other hand, plant-microbial interactions can range from deleterious (pathogenic/host manipulation) to beneficial (mutualistic to symbiotic), but interesting evidence indicates that boundaries in-between are dynamic, where different host fitness-outcomes involve similar principles. Then, using the same study model, we will discuss on how the abiotic environment could shape the consequences of this relationship, potentially transforming the outcome of a plant-bacterium interaction.

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Small, non-coding, regulatory RNAs and RNA-binding proteins in the N_2-fixing symbiont *Sinorhizobium meliloti*

Claudio Valverde[1].
(1) Laboratorio de Bioquímica, Microbiología e Interacciones Biológicas en el Suelo, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes - CONICET, Bernal, Buenos Aires, Argentina.

Sinorhizobium meliloti (*Sm*) is an alpha-proteobacterium that thrives in the soil and that may establish an endosymbiotic lifestyle as a highly differentiated bacteroid devoted to biological nitrogen fixation within the root nodules of alfalfa and related *Medicago* species. From one habitat to the other, *Sm* faces marked changes in gene expression which require fine-tuning at different levels. One major layer of regulation is determined by a genomic complement of over 400 genes for small and non-coding regulatory RNAs of the *trans*-acting class (sRNAs), which are main players in post-transcriptional control of gene expression, often with the assistance of the RNA-binding protein Hfq. However, the regulatory targets of the vast majority of the *Sm* sRNAs have not been elucidated yet. Here, we will summarize the state of the art in this field and present recent findings in the characterization of the biological function of an sRNA gene of *Sm* strain 2011 that belongs to a broadly conserved family of regulatory RNAs of the alpha-proteobacterial class.

Symposium - Cannabinoid and cannabinoid receptors: Molecular Mechanisms and Implications in Medicine

Entourage effect for cannabinoids present in Cannabis sativa at the molecular level and its implication for health

X Steinberg[1,2], F Silva[1], V Vergara[1], C Verdugo[1], E Mendoza[1], FJ Sepulveda[1,2].
(1) Fundación Ciencias para la Cannabis, Concepción, Chile.
(2) Departamento de Bioquímica y Biologia Molecular, Universidad de Concepción, Concepción, Chile.

The Cannabis sativa plant is a vegetable with a long history alongside humans because of its great versatility of uses. Commonly, subspecies of fiber-type cannabis (typically hemp) have been used for the production of textile and construction materials, among others. However, exist other subspecies named narcotic type (typically cannabis or marijuana) at the present widely used for medicinal, social or recreational, and also spiritual purposes. Beyond its botanical characteristics, the difference between the differents strains of Cannabis its in chemical or chemovar composition (from the English “chemovar”). Phytocannabinoids, terpenes, terpenoids, and flavonoids form a large part of the content for common Cannabis strains. Interestingly, the relative proportions of these molecules are found determine their chemotype and consequently their potential for use in humans and other animals.

Phytocannabinoids are the group of molecules with the greatest therapeutic potential, and their pharmacological target in animals is the endocannabinoid system (eCB). This signaling system is widely expressed in many species with different levels of complexity, such as a master regulator of homeostasis processes.

Great efforts have been made from the scientific community to isolate and synthesize cannabinoids separately in order to understand their effects on different models of preclinical and clinical study. However, the effect of isolate cannabinoids looks different to compare with the effect produced by a mixture of different cannabinoids. This phenomenon is called the Entourage effect, and the molecular mechanisms are not understood at present. In this presentation, we will present evidence from our and other research groups related to the entourage effect

Modulation of ionotropic glycine receptors by cannabinoid ligands

Gustavo Moraga-Cid[1], Carlos F. Burgos[1], Jorge Fuentealba[1], Patricio A. Castro[1], Leonardo Guzmán[1], Carola Muñoz-Montesino[1], Hanns U. Zeilhofer[2], Luis G. Aguayo[1], Gonzalo E. Yévenes[1].
(1) Departamento de Fisiología. Facultad de Ciencias Biologicas. Universidad de Concepcion
(2) Institute of Pharmacology and Toxicology, University of Zurich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

Glycine receptors (GlyRs) are anion-selective neurotransmitter-gated ion channels of the pentameric ligand-gated ion channels (pLGICs) superfamily. In the mammalian central nervous system, the enhancement of the chloride conductance through the activation of GlyRs results in a transient hyperpolarization of the membrane potential, which is critical to control the neuronal excitability. Moreover, abnormal glycinergic inhibition accompanies a variety of CNS diseases, such as hyperekplexia, epilepsy, autism and chronic pain. Therefore, allosteric modulation of GlyRs appear as a promising therapeutic strategy for the treatment of CNS diseases. In this context, recent evidences have described groups of new GlyR modulators, including several cannabinoid ligands. Both endocannabinoid (EC) and phytocannabinoid (PC) ligands have attracted interest as GlyR potentiators displaying analgesic effects. However, the molecular sites associated with the functional GlyR-cannabinoid interactions remain controversial. Here, by using biochemical, electrophysiological and bioinformatics, we explored the molecular interactions and between ECs and PCs with different GlyR conformations. Our data show that ECs modulate GlyR with subunit-specificity, displaying both potentiation and inhibition of the glycine-activated currents through different GlyR subtypes. Mutational analysis indicated that the EC-induced potentiation of GlyR was dependent on a single lysine residue (i.e. K385), located on the intracellular domain (ICD). Conversely, these mutations did not affect the PCs modulation of the GlyR activity. Molecular docking studies predict that the polar groups may effectively interact with basic residues located ICD. Our results thus identify a critical molecular determinant for the functional modulation of GlyRs by ECs. In addition, they may contribute to clarify the relevance of the GlyR in the *in vivo* actions of several cannabinoid ligands with negligible activity on G-protein coupled cannabinoid receptors.

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New effects for endocannabinoid signaling in the hypothalamus and the implication of the use of cannabinoids as a potential anti-obesity strategy

FJ Sepulveda[1,2], M Konar[1], A Palma[1], J Rojas[3], F Maurelia[2], C Azocar[2], MA Garcia-Robles[1,4], X Steinberg[1,2].
(1) Departamento Biologia Molecular UdeC
(2) Fundación Ciencias para la Cannabis, Concepción, Chile
(3) Centro de Estudios Avanzados de la Vida UdeC
(4) Departamento de Biologia Celular UdeC

The endocannabinoid system (eCB) is composed of a group of GPCRs (receptors CB1 and CB2, endocannabinoid-producing enzymes (DAGLa for 2-AG, NAPE-PLD for AEA) and endocannabinoids-degrading enzymes (MAGL for 2-AG, FAAH for AEA). Two major endogenous lipid ligand are 2-AG and AEA. The endocannabinoid signaling participates in many important homeostatic processes such as food intake and energy balance, among others. Interestingly, endocannabinoid signaling can be modulated by molecules present in the Cannabis Sativa strains such as THC (agonist) and CBD (alosteric inhibitor).However, the cellular and molecular mechanisms by which the endocannabinoid system acts during eating behavior are not 100% understood. Using western blot and immunohistochemistry (IHC) were performed in basal hypothalamus sections of adult rats under several glycemic conditions to evaluate the sublocalization of DAGLa. qRT-PCR was used to measure hypothalamic neuropeptide levels. Adenoviral particles carrying shRNAi specific for DAGLa mRNA and DALGa inhibitors were used to study the effect on feeding behavior. We foundusing *in vitro* and *in situ* approaches we demonstrated that variations in extracellular glucose concentration produce significant changes in the subcellular localization of DAGLa. II) DAGLa present in tanycytes modulate the levels of neuropeptides produced by orexigenic (NPY) and anorexigenic (POMC) neurons present in the hypothalamic arcuate nucleus (AN). The modification of the subcellular localization of DAGLa in response to glucose in the tanycytes and the modification of NPY and POMC neuropeptides mRNA levels in NA mediated by DAGLa of tanycites strongly suggests a role for the enzyme that produce 2-AG in tanycytes on feeding behavior.

ACCDiS – SBBM Symposium: Translational Cell Signaling and Metabolism

Role of polycystin-1 in the regulation of cardiac function

Francisco Altamirano[1].
(1) Internal Medicine Cardiology, UT Southwestern Medical Center

Autosomal-dominant polycystic kidney disease (ADPKD) is a progressive renal disorder with adult onset. Mutations in *PKD1* or *PKD2,* encoding polycystin-1 (PC1) and PC2, underlie ADPKD. The disease manifests with multiple cardiovascular alterations, including cardiac systolic and diastolic dysfunction. The extent to which these abnormalities stem from the effects of comorbidities impinging on the heart versus cardiomyocyte-autonomous actions of mutant polycystin proteins are unknown. Coassembly of both PC1 and PC2 produces unique cation-permeable channels, which were thought to promote Ca2+ entry at the primary cilia in renal epithelial cells after mechanical stretch. ADPKD patients harbor PC1 and PC2 loss-of-function mutations in their cardiomyocytes, but the functional consequences are unknown. Our research focuses on understanding the role of PC1 and PC2 in cardiac function and contractility. *Pkd2* deletion, exclusively in cardiomyocytes, does not affect systolic nor diastolic function. On the other hand, cardiomyocyte-specific deletion of *Pkd1* impairs systolic and diastolic function in mice. We demonstrated that PC1 ablation reduces action potential duration and sarcoendoplasmic reticulum Ca2+ ATPase (SERCA) activity in cardiomyocytes, secondarily decreasing both Ca2+ transients and myocyte contractility. We linked the effects of PC1 ablation (in cardiomyocytes) to an increase in voltage-gated outward K+ currents, resulting in faster repolarization. Over-expression of full-length PC1 in HEK293T cells significantly reduced the current density of heterologously expressed Kv4.3, Kv1.5, and Kv2.1 channels. In aggregate, our findings uncover a novel role for PC1 controlling action potential duration and SERCA. PC1-deficient cardiomyocytes have impaired contractility, which may explain cardiac dysfunction observed in ADPKD patients.

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Dysfunctional Mitophagy in human Down´s Syndrome induced Pluripotent Stem Cells

Valentina Parra[1,2,3,4].
(1) Department of Biochemistry and Molecular Biology, Faculty of Chemical and Pharmaceutical Sciences, Universidad De Chile, Santiago, CL
(2) Advanced Center for Chronic Diseases, Faculty of Chemical and Pharmaceutical Sciences, Universidad de Chile, Santiago, CL
(3) Center for Exercise, Metabolism and Cancer Studies, Faculty of Medicine, Universidad de Chile, Santiago, CL
(4) Autophagy Research Center, Faculty of Odontology, Universidad de Chile, Santiago, CL

Introduction.
Mitochondria are highly dynamic organelles that undergo different processes of fusion, fission, biogenesis and mitophagy. Perturbation in either direction in normal mitochondrial dynamics can lead to the accumulation of damaged and inefficient organelles. The gene DSCR1 or RCAN1, on the critical Down syndrome (DS) region 1, is an inhibitor of the phospahatase calcineurin that promotes mitochondrial fission through DRP1 dephosphorylation.

Methodology.
We use advanced imaging techniques and siRNA to dissect the role of RCAN1 in mitochondrial dynamics and function of DS induced pluripotent stem cells (iPSCs).

Results.
Trisomic iPSCs showed a decrease in the mitochondrial number per cell, along with an increase in the mitochondrial mean volume in comparison to disomic cells, which is consistent with lower rates of mitochondrial fission. Moreover, DS iPSCs had an increased basal and proton leak-induced oxygen consumption, as well as decreased levels of PINK1 (a protein involved in mitophagy) compared to control iPSCs. The decreased mitochondrial fission and PINK1 protein levels of the DS iPSCs were rescued with a RCAN1 siRNA.

Conclusion.
These data suggest that RCAN1 decreases mitochondrial fission and mitophagy by the inhibition of the calcineurin-dependent activation of DRP1, thus increasing mitochondrial network connectivity.

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The Heart has a Mitochondrial Clock

Beverly Rothermel[1].
(1) UT Southwetern

Many aspects of the cardiovascular system display robust circadian rhythmicity. In humans, damage to the heart from ischemia/reperfusion (I/R) is greatest near the time of waking. The impact of this on human health reaches well beyond events specific to having a heart attack. For instance, the long-term outcomes of surgeries requiring cardiac assist, such as valve replacements, are significantly better for those performed in the afternoon than for those scheduled in the morning. Similarly, mice show an almost two-fold greater sensitivity to reperfusion damage near the time of waking compared to at the end of their active period. Pharmacological inhibition of the protein phosphatase calcineurin (CN) is protective at the time of greatest risk but provides little benefit at other times of day. This suggests that, although I/R causes damage to the heart at any time of the day, there is a CN-dependent component that varies in magnitude depending upon the time of day, and that the mechanism of damage involved is amenable to intervention at the time of reperfusion. We have evidence that this CN-dependent process involves daily cycling of mitochondrial fission and fusion in the heart that translates directly into changes in the capacity for mitochondrial Ca^{2+} uptake via the mitochondrial Ca^{2+} uniporter (MCU). The cycling of mitochondrial fission and fusion is synchronized for maximal ATP generation at times of day when cardiac demand is greatest while providing for continual repair and replacement of mitochondria at times of lower demand. Although this is clearly beneficial for maintaining cardiovascular health over a lifetime, we postulate that it leaves the heart more vulnerable to oxidative damage at the time of day in the cycle when mitochondria are most fragmented, and thus, less efficient at buffering cytosolic Ca^{2+}. Our long-term goal is to develop new approaches to the treatment and prevention of cardiovascular disease that take advantage of the innate circadian rhythmicity of human physiology rather than working against it.

Integrative bioinformatics and systems biology of long non-coding RNAs in non-communicable diseases

Maracaja-Coutinho V [1]
(1) Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile

Long non-coding RNAs (lncRNAs) comprise the most representative transcriptional units of the mammalian genome. Despite the existence of a large number of lncRNAs in the human genome, only the mechanism of action of a small fraction of them is known. However, these cases show that lncRNAs exert a variety of actions in the functional regulation of the genome architecture and gene expression. Moreover, lncRNAs are known to be associated with different human non-communicable diseases, including cancers, neurological disorders and cardiovascular conditions. As envisioned by Dr Walter Gilbert in 1993, a huge volume of different types of genomic and transcriptomic data accumulated during the last two decades in public databases. The proper exploration of this data can be a powerful tool to accelerate data driven discovery, increasing the knowledge behind human biology and disease. In this talk, we will present different computational approaches developed/applied by our group, in order to explore the amount of data available, and its applications in the functional characterization of lncRNAs and associations with different cancer types and cardiovascular diseases. We will also present different cases of study related to the elucidation of a potential mechanism of lncRNAs regulating genomic architecture; their potential relationship with immunological pathways related to vaccination; their putative epitranscriptome regulation by RNA modifying enzymes; and their single-cell expression profiling along heart development.

FONDECYT-CONICYT (11161020), FONDAP-CONICUT (15130011) and PAI-CONICYT (PAI79170021).

New member session

Molecular determinants of HCO_3^- transport of the human AE4 (SLC4A9) Cl^-/HCO_3^- exchanger

Lisandra Flores[2,4], Juan Viveros[2,4], Fernanda Fernandez [2,4], Marcelo Catalán[2], Sebastian Brauchi[2,4], Wendy Gonzalez[3,4], Daniel Bustos[3], **Gaspar Peña-Münzenmayer[1,2,4]**.
(1) Bioquímica y Microbiología, Ciencias, Universidad Austral de Chile, Valdivia, CL
(2) Fisiología, Medicina, Universidad Austral de Chile, Valdivia, CL
(3) Bioinformática, Ingeniería, Universidad de Talca, Talca, CL
(4) Núcleo Milenio de Enfermedades Asociadas a Canales Iónicos (MiNICAD), CL

Introduction:
The AE4 HCO3- transporter plays a role in renal NaCl reabsorption and fluid secretion in salivary glands. It has been proposed that AE4 mediates Cl-/cation–HCO3- exchange, however, residues participating in the transport mechanism are unknown. Our sequence alignments showed that functionally critical residues reported in other SLC4A transporters are conserved in AE4, suggesting that its transport mechanism might be encoded by the same set of residues. The main goal of this study is to identify the residues involved in the transport cycle of AE4.

Methods:
We explored the transport mechanism of AE4 using molecular modeling, site-directed mutagenesis and HCO3- transport assays using imaging techniques.

Results:
We mutated residues D709, T448 and I758 in AE4 and found that D709 and T448 are functionally important. However, in contrast to other SLC4 transporters, our mutants conserved more than 50% of activity. We additionally mutated residues S446, S447, S754 and T756. Mutants S446A and T756A decreased transport activity by ~30% while mutations of S447 and S754 didn´t show functional changes. These results suggest that the individual contribution of mutated residues to the transport cycle is partial. Therefore, we analyzed three double-mutants and found that mutant T756A-T448I abolished transport. Our molecular simulations showed that the ion translocation pathway is narrower in the T756A-T448I mutant, suggesting that the transport cycle is impaired due to structural rearrangements.

Conclusions:
Our results are consistent with a critical role of residues T756 and T448 in the transport cycle of the AE4 exchanger.

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Novel implications of the unfolded protein response (UPR) sensor IRE1α in the control of cell movement: implications into human diseases

Hery Urra[1,3], Limia Celia M[1,2,3], Paloma Moraga[1,3], Raul Aravena[1,3], Eric Chevet[2], Claudio Hetz[1,4,3].
(1) Biomedical Neuroscience Institute (BNI), CL
(2) Chemistry, Oncogenesis, Stress and Signaling, , FR
(3) Center for Geroscience, Brain Health and Metabolism (GERO), CL
(4) Buck Institute for Research on Aging,, US

IRE1α is a crucial signal transducer of the unfolded protein response (UPR). Classical activation of IRE1α under ER stress triggers adaptive and pro-apoptotic signals; however, recent reports have suggested new functions of IRE1α in different physiological processes not related to ER stress. Using an interactome approach we identified putative interaction of IRE1α with several proteins involved with cytoskeleton including Filamin A, an actin-crosslinking protein involved in actin remodeling and cell migration. Using several approaches we validated and mapped the interaction domains of IRE1α and Filamin A. Moreover, IRE1α is involved in Filamin A phosphorylation under ER stress and serum stimulation; phosphorylation that is required for actin remodeling. Functional assay also demonstrated that IRE1α increases actin remodeling and cell migration in mouse embryonic fibroblasts. In addition, using *in utero* electroporation we demonstrated that silencing of IRE1α in the brain cortex of mouse embryos led to retardation of migrated neurons in the cerebral cortex. In addition, we have observed that IRE1 might also impact invasion and metastasis in Glioblastoma and melanoma models, respectively. Therefore, here we uncovered a novel function of IRE1α in actin remodeling and cell migration trough Filamin A impacting brain development disorders and Cancer.

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Incorporation

A new synthetic biology approach allows transfer of an entire metabolic pathway from a medicinal plant to a biomass crop

Paulina Fuentes[1], Fei Zhou[2], Alexander Erban[3], Daniel Karcher[3], Joachim Kopka[3], Ralph Bock[3].
(1) Biología, Ciencias, Universidad de Chile
(2) National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan, CN
(3) Max-Planck-Institut für Molekulare Pflanzenphysiologie, DE

Artemisinin-based therapies are the only effective treatment for malaria, the most devastating disease in human history. To meet the growing demand for artemisinin and make it accessible to the poorest, an inexpensive and rapidly scalable production platform is urgently needed. Here we have developed a new synthetic biology approach, combinatorial supertransformation of transplastomic recipient lines (COSTREL), and applied it to introduce the complete pathway for artemisinic acid, the precursor of artemisinin, into the high-biomass crop tobacco. We first introduced the core pathway of artemisinic acid biosynthesis into the chloroplast genome. The transplastomic plants were then combinatorially supertransformed with cassettes for all additional enzymes known to affect flux through the artemisinin pathway. By screening large populations of COSTREL lines, we isolated plants that produce more than 120 milligram artemisinic acid per kilogram biomass. Our work provides an efficient strategy for engineering complex biochemical pathways into plants and optimizing the metabolic output.

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SchMON1 and *SchCCZ1*, two putative guanine nucleotide exchange factors genes from *Solanum chilense*, confer salt stress tolerance to transgenic Arabidopsis

José Antonio Madrid-Espinoza[1], Josselyn Salinas-Cornejo[1], Simón Ruiz-Lara[1].
(1) Instituto de Ciencias Biológicas, Universidad de Talca, Talca, CL

In plants, endocytosis and pre-vacuolar vesicular trafficking are rapidly induced mechanisms against salt-stress conditions. They allow the reduction of excess sodium in the cytosol, preventing the influx, increasing the efflux, or maximizing its vacuolar compartmentalization. Both mechanisms are activated by Guanine nucleotide Exchange Factors (GEF). In this context, we have identified two *GEF* genes from halophyte-tomato wild relative, *Solanum chilense*, homologs to *CCZ1* and *MON1* from *Arabidopsis thaliana*, denominated *SchCCZ1* and *SchMON1*. Both CCZ1 and MON1 form a heterodimer that first mediates the fusion between endocytic vesicles and late endosome, and then activates RabG-proteins regulating of vesicular traffic towards the vacuole. Herein, we showed that the expression patterns of both genes were early up-regulated in leaves and roots during salt stress in *S. chilense*. In addition, we visualized the subcellular locations of both proteins in *A. thaliana*, using a transient-transformation method. Furthermore, we demonstrated that the individual and co-expression of both *SchCCZ1* and *SchMON1*, under the control of a specific root promoter (At1g73160), enhanced tolerance to salt stress in transgenic *A. thaliana*. This tolerance was consequence of a higher rate of endocytosis, acidification levels and accumulation of sodium in the vacuoles of the root cells. Our results suggest that the endocytosis and vesicular trafficking activated by *SchMON1* and *SchCCZ1* genes in *S. chilense* are mechanisms of protection against salinity stress, capacity that could be transferred to salt-stress sensitive plants.

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Theory and molecular dynamics on F1-ATPase accounting for early events in ADP release

Matute Ricardo A. [1,2].
(1) Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California , US
(2) Centro Integrativo de Biología y Química Aplicada (CIBQA), Universidad Bernardo O´Higgins, Santiago, CL

Single-molecule imaging experiments provide information that is not available from ensemble experiments. We are interested in the interpretation of dynamical studies imaging and manipulation in F1-ATPase single-molecules. One key question that has arisen in single molecule stalling experiments is the erratic behavior a rotor angle of 55° between the binding and hydrolysis dwell angles of 0 and 80°, respectively. We have used an elastic property of the rotor-stator structure to treat the experiments on controlled rotation. Our modelling suggests that there has to be a change in the bonding network, for example, of hydrogen bonds, as the system transitions between the two dwell points, perhaps at 55°, as indicated by an unusual stalling behavior around that angle. In the present work, we performed unbiased MD simulation of the 3-occupancy structure of the F1-ATPase for 650 nanoseconds, running trajectories for both the mono- and the diprotic states of the phosphate. From the analysis of the trajectories, we found that strong electrostatic interaction between the monoprotic phosphate and the Mg ion becomes an effective anchor to keep the ADP in a relatively fixed position. In contrast, the diprotic phosphate is interacting with the ADP through a hydrogen bond, which is disrupted promptly, with a concomitant displacement of the Mg-ADP in around 3-4 angstroms.

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Adenosine metabolism as a therapeutic target for glioblastoma cell invasion

Ignacio Niechi[1], Atenea Uribe-Ojeda[1], Erices José[1], Daniel Uribe[1], Claudia Quezada[1].
(1) Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral De Chile

Glioblastoma is the brain tumor with worst prognosis, mainly due to a cell subpopulation called glioblastoma stem-like cells (GSCs). These cells, produce high levels of adenosine, which are increased even more under hypoxic conditions and is related to HIF-2α expression/stability, thereby enhancing cell invasion. Adenosine can be degraded using recombinant adenosine deaminase (ADA) to revert its pathological effects. The aim of this study is to degrade adenosine using ADA in order to decrease HIF-2α-mediated cell invasion in GSCs under hypoxia. GSCs were prepared from U87 human GBM cell line or primary cultures. Cells were incubated under normoxic (21% O_2) or hypoxic (0.5% O_2) conditions. Adenosine depletion was performed using 1 U/mL of ADA. mRNA and protein levels were measured by RT-qPCR and western blot, respectively. Protein stability was measured by cycloheximide assay. Migration and invasion were measured by transwell and matrigel-coated transwell assay, respectively. The addition of ADA is able to decrease extracellular levels of adenosine in hypoxic GSCs. HIF-2α protein but not mRNA levels decrease under ADA treatment in GSCs. HIF-2α protein stability decreases in treatment with ADA under hypoxia in GSCs which is corelated with Snail and Twist1 downregulation. Cell migration and invasion decrease in GSCs treated with ADA under hypoxia, in U87 cell line and primary cultures. In conclusion, adenosine depletion in GSCs using ADA decreases HIF-2α protein stability under hypoxia conditions, thereby decreasing EMT-markers expression and cell invasiveness.

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Protein levels and optogenetic control for horizontally acquired genes in yeast revealed hallmarks of adaptation to nitrogen-limited environments

Francisco Salinas[1,2,3].
(1) Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, CL
(2) Millennium Institute for Integrative Biology (iBio), CL
(3) Centro de Estudios en Ciencia y Tecnología de los Alimentos (CECTA), Universidad de Santiago de Chile, Santiago, CL

In the past decade, sequencing large cohorts of *S. cerevisiae* revealed the landscape of genomic regions acquired by Horizontal Gene Transfer (HGT). The horizontally acquired genes have shown to play important roles in yeast adaptation to the fermentation process, improving nitrogen and carbon sources utilization. However, the functional characterization of these genes at the molecular level has been poorly attended. In this work, we performed a systematic analysis of the promoter activity and protein expression level for 30 genes contained in three horizontally acquired regions (A, B and C). In three strains (one for each region), we used the luciferase reporter gene and the *mCherry* fluorescent protein to quantify the transcriptional and translational activity of those genes, respectively. We assayed the strains in four different culture conditions, showing low levels of transcriptional and translational activity across environments. However, we observed an increase in protein levels under low nitrogen culture conditions. Furthermore, since the strains carrying the luciferase reporter gene are null mutants for the horizontally acquired genes. We assayed the fermentation kinetic and growth parameters (latency time, growth rate and efficiency) in this set of deletion strains. The results showed that four horizontally acquired genes influenced the fermentation rate (Vmax) and fifteen of them affected the growth parameters. Additionally, we performed the overexpression of the horizontally acquired genes through optogenetic control, reveling a gene within region B involved in yeast flocculation. Altogether, our results provided molecular and phenotypic evidence highlighting the importance of HGT in yeast adaptation to nitrogen-limited environments.

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Comunicación Libre Oral

Identifying the interactions between natural, non-caloric sweeteners and the human sweet receptor by molecular docking

Waldo Acevedo[1,2], César Ramírez-Sarmiento[3], Eduardo Agosin[2].
(1) Institute of Chemistry, Faculty of Science, Pontificia Universidad Católica de Valparaíso, Valparaíso, CL
(2) Department of Chemical and Bioprocess Engineering, School of Engineering, Pontificia Universidad Católica de Chile, Santiago, CL
(3) Institute for Biological and Medical Engineering, School of Engineering, Pontificia Universidad Católica de Chile, Santiago, CL

Natural sweeteners exert their sweet taste by specifically binding to sweet taste receptors. However, the molecular basis of their sweetening ability remains to be ascertained. Here, we predicted the sweetening capacity of different families of natural sweeteners – from glycosylated terpenoids, e.g. stevia or monk fruit, to sweet proteins, e.g. monellin or thaumatin – using molecular simulations.To this end, we built comparative models of the hT1R2 and hT1R3 subunits of the sweet taste receptor, using the metabotropic glutamate receptor as a template. Once these models were obtained, we identified the potential binding sites and estimated the binding free energies and interactions of 29 different sweeteners via molecular docking. The binding free energy between hT1R2-hT1R3 and sweeteners of different families showed a strong correlation with their sweetness intensity for both, small sweeteners (r=−0.89) and sweet proteins (r=−0.97). The correlation is further improved and generalized throughout all families of sweeteners evaluated, when EC50 values are used instead of relative intensities (r=−0.91). Altogether, these results contribute to a better understanding of the sweetness perception of these sweeteners, and promote the use of docking for better pre- diction of resulting sweetness.

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The endoplasmic reticulum master regulator protein BiP acts as a ratchet molecular motor in translocation

Hilda M. Alfaro-Valdés[1], Karina New[1], Robert Lesch[2], A.M. Christian Wilson [1].
(1) Biochemistry lab, Biochemistry and Molecular Biology department, Faculty of Chemistry and Pharmaceutical Sciences, Universidad De Chile, Santiago, CL
(2) Department of Molecular and Cellular Biology, Howard Hughes Medical Institute, University of California, , Berkeley, CL

Post-translational protein translocation through the endoplasmic reticulum is mediated by a channel called Sec61 complex and an auxiliary motor protein called BiP (Immunoglobulin Binding Protein). The force exerted by BiP has not been completely elucidated and studies suggest that BiP could be involved in a passive rectification mechanism of the movement by Brownian motion (ratchet model) and/or possibly in an active mechanism of direct pulling (power stroke model). To answer this question, we have developed a novel methodology to study forces in bulk. This aims to determine if folded proteins with known unfolding forces, as measured by optical tweezers, could cross microsomal membranes by BiP-mediated unfolding of the protein. We prepared different constructs that included signal peptide, unfolded titin (to allow entry into the microsomes), and the folded protein, and then used these in our translocation assays. First, a 2-titin chimera protein construct was used as a positive control in the protection test, and could be translocated into microsomes with an efficiency of 31% . Subsequently, the negative control of the Top 7 chimera protein construct proved not to be translocated due to the high unfolding force of this protein (around 35 pN), which is above the level of force that a molecular power-stroke engine could generate. Finally, the CaM chimera protein (unfolding force of around 7 pN) also was shown not to be translocated, thus suggesting that the BiP protein would act as a ratchet-type molecular motor in post-translational protein translocation.

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Influence of poly (dA:dT) tracts and a transcription factors on nucleosome remodeling activity of ISW1a

Roberto Amigo[1], Camila Rivas[1], Gutiérrez José L[1].
(1) Departamento Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, CL

Nucleosome landscape can influence different processes, such as transcription. Thus, to know the nucleosome positioning patterns in regulatory regions has become relevant to understand the mechanisms underlying this process. Numerous studies have found that many gene promoters are characterized by having a long DNA region depleted of nucleosomes (around 150 bp) called nucleosome-depleted regions (NDRs). How these regions remain without nucleosomes is not yet fully understood. ATP-dependent chromatin remodeling complexes appear as important player in NDR formation. While SWI/SNF subfamily members have been associated to NDR maintenance, ISWI subfamily members have been shown to contribute to equal spacing between nucleosomes. Consistently, it has been found that ISW1a can close an NDR when RSC (SWI/SNF member) is not present. In addition, it has been found that transcription factors can function as barrier for sliding activity of ISW1a complex. Besides binding sites for TFs, promoters with NDRs usually contain poly (dA:dT) tracts. However, the role of these sequences in the remodeling activity of these complexes is poorly understood. In order to address the role of these sequences in this aspect of chromatin dynamics, we used *in vitro* studies to determine the influence of poly (dA:dT) tracts on nucleosome remodeling activity of ISW1a complex. In addition, we performed assays using Gal4-DBD to determine if there is a synergistic action of TFs and poly (dA:dT) tracts. We found that poly (dA:dT) tracts can function as barrier for sliding activity of the ISW1a complex.

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Physiological and molecular characterization of grain response to heat stress in different wheat ploidies

Anita Arenas-M[1], Francisca Castillo[1], Daniel Calderini [1], Javier Canales[2].
(1) Instituto de Producción y Sanidad Vegetal, Facultad de Ciencias Agrarias, Universidad Austral De Chile, Valdivia, CL
(2) Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral De Chile,, Valdivia, CL

In a climate change scenario, the crops yield will be affected by the increase of mean global temperature, but also by more frequent extreme heat events like heatwaves. Wheat is an essential crop in the global food security because supplying 20% of the total calories consumed by the human population. High temperatures during grain-filling stage could impact on yield-components, mainly grain weight and grain quality. The aim of this work was to assess the response of the grain in wheats of different ploidy to high temperature conditions during the key stages of grain development. Hexaploid, tetraploid and diploid wheats were sown in field during two consecutive seasons. Ten days after flowering, plants were subjected to four days heat stress and then returned to ambient conditions until physiological maturity. Heat stress decreased individual grain weight and grain dimensions across wheat ploidies. Grain quality traits such as starch and protein content were modificated in a specific way for each species. Transcriptomic analysis of heat stressed grains under field conditions allowed us to identify 6043 1727 and 1509 significantly expressed genes in hexaploid, tetraploid and diploid wheat respectively. In addition, gene co-expression network analysis identified gene modules correlated with physiological yield components and highly connected transcription factors of the Myb, Dof and Heat shock TF families with target genes related to RNA processing, glycogen metabolism and heat acclimation.

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Carotenoid Biosynthesis in carrot: regulatory role of light regulated genes

Daniela Arias[1], Angélica Ortega[1], Jonathan Canales [1], Christian González[1], Felipe Durán [1], Claudia Stange [1].
(1) Centro de Biología Molecular Vegetal, Facultad de Ciencias, Universidad de Chile, Santiago , CL

Carotenoids are pigments that provide yellow, orange and red colors to flowers, fruits and some vegetables. They contribute to light-harvesting and photoprotection during photosynthesis, serve as scavengers for oxidative damage and are essential for phytohormone synthesis. Carotenoid synthesis is induced by light during plant development (photomorphogenesis) and fruit ripening. *Daucus carota (carrot)* synthesizes and accumulates large amounts of carotenoids in its storage root grown in dark and contrary to other plants, light inhibits the synthesis of these pigments and storage root development. To understand the molecular processes that regulate carotenoids biosynthesis in the carrot root, we generated an RNA-Seq analysis between the root grown in light (R/L) and darkness (R/D). Unexpectedly, genes involved in Shade Avoidance Syndrome (SAS) and photomorphogenesis such as *DcPAR1* and *DcPIF3* were up-regulated in R/D and down-regulated in R/L. In SAS, AtPAR1 interacts with and inhibits AtPIF, avoiding excessive hypocotyl elongation. *AtPAR1* also promotes carotenoid synthesis while *AtPIF1* represses the expression of carotenogenic genes in Arabidopsis. Here, we determined that DcPAR1 and DcPIF3 interact *in vivo* and their expression in carrot root development correlates with carotenoid synthesis. The functional characterization of *DcPAR1* and *DcPIF3* was achieved byoverexpression and/or silencing in Arabidopsis and carrot, resulting in a dramatic effect in plant development, together with changes in the expression levels of key carotenogenic genes and in carotenoid content. Our results indicate that *DcPAR1* and *DcPIF3* codify for functional factors that participate in photomorphogenesis and carotenoid synthesis.

Proyecto Fondecyt 1180747

Studying the changes of thermal stability of a protein throughout its evolutionary history using ancestral sequence reconstruction

Cea Pablo A. , Rodrigo Recabarren[1], Maturana Pablo[2], Jans Alzate-Morales[1], Victoria Guixé[2], Victor Castro-Fernandez[2].
(1) Centro de Bioinformática y Simulación Molecular, Facultad de Ingeniería, Universidad de Talca, Talca, CL
(2) Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, CL

Natural selection has tuned the molecular machinery of living organisms to carry out their functions even in the harshest environments. Within this context, proteins from extremophilic organisms have acquired a wide variety of adaptations to remain folded under conditions that would normally lead to denaturation, like extreme salinity and temperature. In this work, we studied protein thermal stability from an evolutionary perspective, using Ancestral Sequence Reconstruction. Through this approach, we reconstructed the divergence process between bacteria from the thermophilic Thermales and the mesophilic Enterobacteria orders using the enzyme hydroxymethyl pyrimidine kinase (EC. 2.7.1.49) as a model system. The in vitro characterization of these enzymes revealed that thermal stability follows a clear divergent trend; the thermal stability increases along with the evolution of the thermophilic lineage, whereas, it was lost in the mesophilic branch. To analyze the atomic details underlying these remarked differences, we modeled and performed molecular dynamics simulations of the different proteins studied. Our results show that the trend in thermal stability is correlated with conformational flexibility, that is, during evolution toward less thermotolerant forms the flexibility increases and vice versa. Also, we inspected how electrostatic interactions and hydrophobic contacts evolved in both branches. Both types of interactions increase in number in the thermophilic lineage compared to the mesophilic branch, moreover, details regarding the optimization of these interactions will be discussed. Overall, our results suggest that evolution modulates the conformational dynamics and stability of a protein through the fine-tuning of its non-covalent interaction network.

Fondecyt de Iniciación 11181133

bZIP25 as a negative regulator in endocytic trafficking and a modulator in lateral root development in response to nutritional deficit

Damián Cifuentes Aguilar[1], Francisco Pavez Malmborg[1], Claudio Osorio-Navarro[1], Lorena Pizarro[1], Lorena Norambuena[1].
(1) Department of Biology, Faculty of Sciences, Universidad de Chile, Santiago , CL

Endomembrane system comprises a complex network of functionally connected compartments and vesicles. In our laboratory, we have proposed endocytosis as part of the process of lateral root development. In that context, we are interested in studying the molecular regulation in cell trafficking. We have found that the leucine zipper transcription factor bZIP25 is involved in endocytosis, regulating it negatively. The loss-of-function mutant *bzip25-2,* in which the transcript level is diminished lower than 90%, displays a higher rate of endocytosis. There are three *bZIP25* mRNA isoforms in Arabidopsis, however, the role of each them is unknown. In order to determine their role in the endocytosis process, bZIP25.1, and bZIP25.2 isoforms were expressed in the *bzip25-2* mutant as GFP-fusion protein. We found that each one separately rescued the mutant phenotype, indicating that both isoforms participate as negative regulators of endocytosis. To investigate whether bZIP25 and the mRNA isoforms would have a role in the response to nutritional status of plants, the root architecture was analyzed when plants are under low availability of phosphate or nitrogen. We found that bZIP25.2 has a role in lateral root development in response to phosphate deficiency. Taken together, these evidences would point bZIP25 not only as a negative regulator of endocytosis but also as a participant in the phosphate nutritional deficit response.

Fondecyt 1170950

Electrical signaling in plants

Ingo Dreyer[1].
(1) Centro de Bioinformática y Simulación Molecular (CBSM), Universidad de Talca

Long-range signaling in higher organisms is essential for flexible responses to any environmental threat, challenge or stimulus. Animals have a nervous system for this purpose with a dense network of nerve cells connected via their axons and synapses that allows fast transmission of action potentials. These are electrical signals propagating from one part of the body to another. In contrast to animals, higher plants do *not* have a nervous system. Nevertheless, they do operate long-distance electrical signaling, too. Prominent and very illustrative examples are the sensitive plant leaves folding of *Mimosa pudica* in response to touch and the closure of the Venus flytrap *Dionaea muscipula* when stimulated by an insect. But also, the communication of an herbivore attack from one leaf to other parts of the plant is mediated by electrical signals. The cellular and molecular nature of electrical signaling in plants and the underlying 'green circuits' are still poorly understood. In recent years, however, new insights into electrical signaling in plants could be obtained. It was shown that potassium channels can influence the propagation of electrical signals at the plasma membrane and that a network of vacuolar channels determines the excitability of the tonoplast (Jaslan et al., 2019, Nat.Comm.). On the conference, an updated view on electrical signaling in plants will be provided.

The Apoptotic Mitochondrial Protein FAM162A has a New Role in Mitophagy and It is Essential for Erythropoiesis

Andrea Matamoros[1], Amori Malleda[1], Marcelo Muñoz[1], Gomez Gabriela Edith[1], **Alvaro A. Elorza[1,2]**.
(1) Institute of Biomedical Sciences, Faculty of Medicine and Faculty of Life Sciences, Universidad Andrés Bello
(2) Millennium Institute on Immunology and Immunotherapy, CL

FAM162A is a mitochondrial protein previously described as a hypoxia-responsive proapoptotic molecule able to activate the intrinsic apoptotic pathway. As a result of bioinformatics analysis, it was determined that fam162a has an mRNA expression pattern similar to NIX during erythroid differentiation. NIX is a mitochondrial protein involved in mitophagy that interacts directly with the autophagic protein LC3 through its LIR domain, and FAM162A has two LIR domains. Terminal erythropoiesis is characterized by extensive mitophagy to clear all mitochondria out and generate a mature red blood cell. It is hypothesized FAM162A has a new role associated with mitophagy and erythropoiesis. Loss of function experiments by knocking down FAM162A in COS-7 cells showed a fragmented mitochondrial network, with low membrane potential and reduced oxygen consumption rate. In addition, a greater number of lysosomes and autolysosomes were observed as well as a decrease in cell viability, suggesting that mitophagy is being arrested when FAM162A is not in mitochondria. Induction of erythroid differentiation in the erythroleukemic cell line K562 increases progressively the expression of FAM162A, which correlates with hemoglobin biosynthesis and the expression of LC3. Consequently, disruption of FAM162A arrested both hemoglobin biosynthesis and erythropoiesis, forcing cells to remain in immature stages. These results suggest that FAM162A has a new role in mitophagy, and it is essential for erythropoiesis.

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Analysis of the protein oligomeric state of dynamic plastoglobule lipoprotein particles of Arabidopsis thaliana

Roberto Espinoza-Corral[1], Peter Lundquist[1].
(1) Department of Biochemistry and Molecular Biology, Plant Resilient institute, Michigan State University , East Lansing, US

The photosynthetically active thylakoid membranes of plant chloroplasts contain associated lipoprotein particles called plastoglobules. These plastoglobules are derived from – and remain attached to – the thylakoid, comprising a distinct population of protein and lipid. While their importance to plant growth and development is highlighted by their dynamic morphology under stresses or developmental transitions and their ubiquity amongst photosynthetic species, their precise role(s) remain unclear. We suggest that the plastoglobule serves in part as a platform for metabolic channeling by drawing pathway enzymes into close physical proximity. To test this hypothesis, we have compared the oligomeric state of the A. thaliana plastoglobule proteome under unstressed and light-stressed conditions by separating proteins by BN-PAGE and analyzing gel slices of increasing native molecular mass by mass spec-based proteomics. We identify multiple proteins present in high molecular mass complexes and propose interactions amongst several plastoglobule proteins based on co-migration on the BN-PAGE gel. Comparison of the two environmental conditions reveals a dynamic oligomeric state, suggesting creation and formation of distinct complexes in response to differing requirements of the plant. We selected a protein kinase that migrates in a high molecular weight complex specifically under light stress for T-DNA mutant studies. Characterization of the mutant demonstrates impairment in the transition from vegetative to reproductive growth, emphasizing the plastoglobules role in developmental transitions. In vitro kinase assays reveal critical residues required for kinase activity and phenotypic complementation. We propose a model of plastoglobule function that synthesizes our discoveries.

Michigan State University

Conformational sampling and polarization of Asp26 in pKa calculations of thioredoxin

Aharon Gomez[1], Esteban Vöhringer-Martinez[1].
(1) Departamento de Fisicoquímica, Facultad de Ciencias Químicas, Universidad de Concepción, Concepción, CL

To study the pKa variation in aspartate residues Thioredoxin protein has been used as model system in several computational methods. An hydrophobic cavity surrounds aspartate residue 26 (Asp26) which make its pKa 3.5 units higher than a solvent exposed one (eg, Asp20). Here, we use extensive atomistic molecular dynamics simulations for two different protonation states of Asp26 in combination with conformational analysis based on RMSD clustering and principal component analysis to identify representative conformations of the protein in solution. For each relevant conformation, proton transfer Gibbs free energy between Asp26 and Asp20, which is fully solvated in a loop region of the protein, are calculated with the Amber99sb force field in alchemical transformations. The varying polarization of the two residues in different molecular environments and protonation states is described by polarized, Hirshfeld-I (HI) atomic charges obtained from the averaged polarized electron density are used. Our results shows that the proton transfer Gibbs free energy depends on the protein conformation, on proper sampling of the neighboring Lys57 residue orientations and on the modified solvation of Asp26 in the deprotonated state when water molecules penetrate the hydrophobic cavity. The inclusion of the polarization of both aspartate residues in the free energy cycle corrects the results from the non-polarizable force field reproducing the experimental ΔpKa value of Asp26.

Fondecyt 1160197

The transcription factor MYB75 modulates growth and the response to sulfate starvation in *Arabidopsis thaliana*

Carlos Henríquez-Valencia[1], Diego Landaeta[1], Javier Canales[1,2].
(1) Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, CL
(2) Millennium Institute for Integrative Systems and Synthetic Biology (MIISSB), CL

Introduction:
Sulfur is an essential macronutrient for plant growth and development. It is well known that genes involved in the sulfate assimilation and metabolism are transcriptional regulated by sulfur demand. However, little is known about the transcription factors that regulates these genes in response to changes in sulfate availability. MYB75, is a regulator of anthocyanin biosynthesis. Interestingly, it has been reported that *MYB75* is induced by sulfate starvation. However, the role of MYB75 in the response to sulfate availability is unknown.

Methodology:
We used plants with gain and loss of function for MYB75. We evaluated several parameters related with the growth, internal sulfate level, anthocyanin content and the gene expression of marker under sulfate availability. Moreover, a transcriptomic analysis was performed from shoot wild-type and over-expressor plants grown under sulfate starvation.

Results:
Over-expression of MYB75 results in a decreased growth under -S conditions, and plants with loss of function showed an increased shoot growth compare with wild-type plants. We found that MYB75 regulate the mRNA levels of enzymes associated with sulfate primary metabolism such as *APRs,* controlling the internal sulfate content in the shoot. The anthocyanin content together with the expression of enzymes involved in the biosynthesis anthocyanins are regulated by MYB75 under -S conditions. The RNA-seq analysis revealed that MYB75 is involved in carbon metabolisms, stress abiotic response and hormonal signaling under sulfate starvation.

Conclusions:
Our data shows that MYB75 plays an important role in plant growth and regulation of sulfate metabolism in *Arabidopsis thaliana*.

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Core proteome of cervico-vaginal fluid of healthy Chilean women at fertile age

Mauricio Hernandez[1], Guillermo Nourdin[1], Alan Fritz[1], Francisco Alvarez[1], Pablo Saldivia[1], Juan Stecher[2], Cristian Vargas[2], Elard Koch[1].
(1) Division of Proteogenomics, MELISA institute, San Pedro de la Paz, CL
(2) Clinical Protocols, Division of Epidemiology, MELISA institute, San Pedro de la Paz, CL

Cervico-vaginal fluid has important function in the homeostasis and immunity of the lower female genital tract. Additionally, cervico-vaginal fluid has great potential as a source of biomarkers for female reproductive tract disease. Current evidence about the proteomic characterization of this fluid does not allow an exact baseline to be determined for the study of biomarkers due to the high variability of techniques used, cohort samples from non-homogeneous patients, a small number of patients studied. Our research group has developed a clinical protocol for obtaining biological samples of cervico-vaginal fluid from a cohort of healthy fertile age chilean women, which has allowed us to carry out proteomics studies with the aim of characterizing the proteome core of this fluid in healthy chilean women. For this study 96 samples of vaginal cervical fluid from 20 patients in the mid-luteal phase were used, carrying out protein extractions using lysis buffer, the digestion of the proteins with trypsin was performed and the peptide mixture was analyzed in the TimsTofPro mass spectrometer coupled to nano elute nanoHPLC (Bruker Daltoniks). A total of 8291 proteins have been identified in all experiments performed. Establishing a Core Proteome composed of 876 proteins. Additionally, we found OVGP1, a specific fallopian tube marker protein. What gives us evidence that vaginal cervical fluid is a source of markers of the upper female reproductive system. Further characterization of cervico-vaginal fluid proteins would contribute to a better search the new proteomics biomarker in the gynecological and obstetrics disease.

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The TGA2 transcription factor negatively regulates the accumulation of the defense hormone Salicylic Acid in *Arabidopsis thaliana*

Ariel Herrera-Vásquez[2,1], Tomás Urzúa[2,1], Aldo Seguel[2,3], Loreto Holuigue[3], Francisca Blanco-Herrera[1,2].
(1) Centro de Biotecnología Vegetal, Ciencias de la Vida, Universidad Andres Bello, Santiago, CL
(2) Instituto Milenio de Biologia Integrativa (iBio), CL
(3) Genetica Molecular y Microbiología, Ciencias Biologicas, Pontificia Universidad Católica de Chile, Santiago, CL

Salicylic acid (SA) is a critical hormone that modulates changes in gene expression inducing the defense response in plants. TGA class II transcription factors that include the TGA2, TGA5, and TGA6 proteins, are essential mediators for transcription of SA-controlled genes. We found on stress conditions, that the lack of TGA2 class II genes (*tga256* plants) show increased expression of genes involved in SA accumulation compared to wild type (WT) plants. To corroborate the participation of TGA class II on the SA-biosynthetic pathway, plants were irradiated with UVC, a well-known SA-inductor stress condition or inoculated with the avirulent pathogen *Pseudomonas syringae* AvrRPM1. Compared to WT, the *tga256* plants show a significant increase in the expression of genes involved in SA accumulation *(PAD4, EDS1, SARD1, ICS1,* and *EDS5).* The same effect was observed at the ICS1 protein level, the main enzyme in SA biosynthesis under stress. The constitutive expression of TGA2 fused to the V5 tag in the *tga256* genetic background rescue the expression of the mentioned genes to a WT state. The quantitation of SA in response to stress indicates that the *tga256* mutant plants show a higher hormone accumulation of the under UVC treatments and pathogen infections. Furthermore, we found that the increase in the SA accumulation in the *tga256* plants is given by the IC pathway. Together, these results suggest that TGA class II factors negatively control SA production under stress conditions.

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The role of host proteins and their post-translational modifications on HIV-1 IRES-mediated translation initiation

Marcelo López-Lastra[1], Valeria Olguín[1], Aldo Barrera[1], Jenniffer Angulo[1], Hade Ramos[1], Leandro Fernández[1], Nataly Contreras[1], Jorge Vera-Otarola[1], Karla Pino[1], Fernando Lowy[1].
(1) Laboratorio de Virología Molecular, Departamento de Infectología e Inmunología Pediatrica, Medicina, Pontificia Universidad Católica De Chile, Santiago, CL

The human immunodeficiency virus type 1 (HIV-1) genomic mRNA harbors an internal ribosome entry site (IRES) within its 5' untranslated region (HIV-1 IRES). Translation initiation mediated by the HIV-1 IRES requires the participation of cellular proteins, IRES trans-acting factors (ITAFs). ITAFs modulate, enhance or repress, translation initiation driven by the HIV-1 IRES. In a previous report, we used a proteomic approach to identify cellular proteins that interact with the 5'UTR of the HIV-1 mRNA, and proposed that some could act as ITAFs for the HIV-1 IRES. In this study, we sought to validate our prediction. Results confirm that the heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1) is positive ITAF for HIV-1 IRES, while the human antigen R (HuR) protein is a negative modulator of HIV-1 IRES activity. Also, by combining UV-CLIP/q-RT-PCR assays, overexpression assays, and endogenous protein knockdown, we identify hnRNPU and hnRNPK as ITAFs for the HIV-1 IRES. Through co-immunoprecipitation assays, we show that hnRNPU, hnRNPA1, hnRNP K, and HuR interact, most probably forming a complex. We select hnRNPA1 as a model ITAF to evaluate the impact of post-translational modifications on the ability of the protein to promote the stimulation of the HIV-1 IRES activity. Results show that phosphorylations and methylations modulate the ability of the protein to act as an ITAF for the HIV-1 IRES. In summary, in this study, we identify cellular proteins that act as ITAFs for the HIV-1 IRES and show that their ITAF activity is modulated by post-translational modifications.

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Identification of long non-coding RNAs and microRNAs in the Atacama Desert fish *Orestias ascotanensis*

Rodrigo Maldonado[1,2], Gino Nardocci[1,2], Martin Montecino[1,2], Miguel Allende[3,2].
(1) Instituto de Ciencias Biomedicas, Universidad Andrés Bello, Santiago, CL
(2) FONDAP Center for Genome Regulation, CL
(3) Department of Biology, Faculty of Sciences, Universidad de Chile, Santiago, CL

Non-coding RNAs including microRNAs (miRNAs) and long non-coding RNAs (lncRNA) have emerged as relevant post-transcriptional regulators of gene expression. Specifically, lncRNAs-miRNA interactions can influence protein expression by the sequestration of a shared miRNA pool that would otherwise have targeted a protein-coding mRNA.

Transcripts involved in such interactions are named competing endogenous RNA (ceRNA).

In this project we aim to determine whether ceRNA interactions contribute to the changes in gene expression that occur during seasonal acclimatization in the Atacama Desert fish Orestias ascotanensis. To achieve this, we have deep sequenced the transcriptome of O. ascotanensis. Prediction of miRNA-mRNA interactions showed a high abundance of transcripts with few miRNA binding sites (MBS) and a small population of transcripts with multiple MBS. Based on their genomic location we found 1,275 (57.5%) lncRNAs with an intergenic location and 942 (42.5%) with a genic location. In addition, we assessed whether widely-conserved lncRNAs that have been shown to have role in establishing lncRNA-miRNA regulatory networks are also expressed in O. ascotanensis. Our results indicate that lncRNAs like Cyrano (oip5-as), including its miR-7 binding site, are conserved in *O. ascotanensis*, albeit its genomic position respect to the nearest protein-coding gene has changed from intergenic to genic. As the function of lncRNAs can be conserved between species despite the lack of sequence similarity, we propose that miRNA-lncRNA interactions that modulate gene expression may have been conserved in *O. ascotanensis*, and that these regulatory networks may have a critical role during adaptation of this fish to its extreme environmental conditions.

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An RNA hub regulating gene expression by long non-coding RNAs

Sara Wernig-Zorc[1], Uwe Schwartz[1], Gernot Laengst[1], **Rodrigo Maldonado[2]**.
(1) Universitaet Regensburg, DE
(2) Laboratorio de Biología Celular y Molecular Aplicada, Universidad Mayor, Temuco, CL

Long non-coding RNAs (lncRNAs) functionally interact with chromatin, regulating gene expression and modifying the nuclear architecture. This interaction can be direct via R-loops (ssRNA:ssDNA) or triple helix structures (ssRNA:dsDNA), or indirectly though chromatin-binding proteins. Nevertheless, the molecular mechanism underlying the lncRNAs-chromatin interaction and the consequent modulation of the gene expression is still poorly understood. Here we knocked-down specific chromatin-associated RNAs (caRNAs) found by chromatin RNA immunoprecipitation (ChRIP-seq), associated with active (MALAT1, NEAT1) and inactive chromatin states (HOTAIR), and combined ATAC-seq and RNA-seq data. This, in order to analyze how those caRNAs affects genome-wide the chromatin accessibility and transcriptome profiles. The data show the existence of a common region located downstream the *NR4A1* gene, where the down-regulation of different lncRNAs affects its accessibility in the same manner. However, when the gene expression levels of the neighboring genes were analyzed by real-time PCR, opposite effects were observed depending on which lncRNA was knocked-down. Several triplex targeting sites are present on this region, which in addition to our results led us to propose the existence of an RNA hub. On this region, different lncRNAs could interact with the chromatin, defining its accessibility levels to subsequently regulate the expression of target genes.

Grape berry cuticle characterization and its possible role in the resistance against *Botrytis cinerea*

Carlos Meyer-Regueiro[1], Armijo Grace I[1], Ulrich Hildebrandt[2], Markus Riedere[3], Patricio Arce-Johnson[1].
(1) Genética Molecular y Microbiología, Ciencias Biológicas , Pontificia Universidad Católica De Chile, Santiago, CL
(2) Julius-von-Sachs-Institute for Biosciences, University of Würzburg, Würzburg, DE
(3) Julius-von-Sachs-Institute for Biosciences, Universität Würzburg, Würzburg, DE

The necrotrophic fungus *Botrytis cinerea* is one of the main grape pathogens, affecting principally ripe berries. It has been reported that the anatomical features such as cuticle and wax content are positively correlated with resistance against this fungus in grape berry. In our lab, we analyzed the susceptibility to *B. cinerea* cultivars Thompson seedless (TS) and the hybrids cultivar Fwe Fuky (FF). TSberries were susceptible to the fungus, whereas FFshowed high resistance. Based on these findings and to assess the possible role of the cuticle in the berry defense against *B. cinerea* we studied the cuticle composition of these cultivars to identify possible features associated with the resistance. In order to understand the difference between them, we focused on the cuticle structure, and composition analyzed by scanning electron microscopy and GC-MS. The composition of the epicuticular layer revealed that FF cultivars present higher concentrations of primary alcohols; the intracuticular layer analysis revealed that in the FF the concentration of two triterpenoids was higher in comparison with TS. The ultrastructure of the epicuticular layer of this cultivar showed a higher density of the palettes crystal compared to TS with higher hydrophobicity. Finally, the possible protective role of this structure was evaluated *in vivo* by disrupting this layer and inoculating the berries. Our results showed that alterations of the ultrastructure enhance the susceptibility of both cultivars. Together, our work suggests that the cuticle is an important structure in the interaction with *B. cinrea* with a protective role in this interaction.

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Differences in structural dynamics of bacterial NusG and RfaH transcription factors upon binding to transcription elongation complexes

José Alejandro Molina[1], Steve Silletti[2], Irina Artsimovitch [3], Komives Elizabeth A.[2], Ramírez-Sarmiento César A.[1].
(1) Instituto de Ingeniería Biológica y Médica, Facultad de Ingeniería, Medicina y Ciencias Biológicas, Pontificia Universidad Católica De Chile, Santiago, CL
(2) Department of Chemistry and Biochemistry, University of California San Diego, La Jolla, California, US
(3) Department of Microbiology and The Center for RNA Biology, The Ohio State University, Columbus, Ohio, US

NusG transcription factors participate in increasing RNA polymerase (RNAP) processivity for most bacterial genes and in coupling transcription to translation. RfaH, a specialized sequence-dependent paralog, is the only NusG member with two native states: its C-terminal domain (CTD) can be folded either as an autoinhibiting α-helical state or, upon recruitment to RNAP paused in ops-DNA sequences, as a β-barrel. Only upon refolding of the CTD into the NusG-canonical β-barrel, the N-terminal domain (NTD) can bind to RNAP to facilitate transcription and translation. Crystallographic and cryoEM studies of NusG and RfaH bound to transcription elongation complexes (TEC) deciphered their interaction modes. However, these static structures do not provide information regarding the differences in regulation mechanisms and conformational changes between these factors. Here we present structural dynamics studies of free and TEC-bound NusG and RfaH using hydrogen-deuterium exchange mass spectrometry to elucidate their regulation mechanisms. NusG-NTD is mainly protected when complexed with TEC, binding to the central cleft in RNAP. RfaH-NTD also showed protection against deuteration, but regions covering β-loop and residues interacting with ops-DNA show higher deuteration when RfaH is complexed with TEC. Finally, NusG-CTD was protected in regions that, according to crystals, would be exposed to tether the ribosome. Conversely, RfaH-CTD showed higher exposure throughout this domain. In conclusion, we found notorious differences between RfaH and NusG complexed with TEC. Dynamic studies of both factors allow us to relate conformational changes upon TEC binding with their regulatory mechanism, suggesting that these differences are attributable to the fold-switching ability of RfaH.

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Evaluating the relationship between different physiological properties and related gene expression in four cultivars of *Fragaria ananassa*

Luis Morales-Quintana[1], **Patricio Ramos[2]**, Carolina Parra-Palma[2], Castro Ricardo I.[1], Cristina Ubeda[1], Mariona Gil[1], Carlos Figueroa[2].
(1) Instituto de Ciencias Biomédicas, Facultad de Ingeniería, Universidad Autónoma de Chile, Talca, CL
(2) Instituto de Ciencias Biologicas, Universidad de Talca, talca, CL

Strawberry is one of the most widely consumed fruits in the world, but under certain environmental conditions the fruit exhibits inadequate red color development, causing economic losses due to lower product quality. In order to evaluated if changes in color is cultivar-specific and environmental-dependent, a comparative study of anthocyanin accumulation, total phenolic content, and total flavonoids analysis was performed, in addition to a transcriptional profile analysis of pigment-related genes in four strawberry (*Fragaria x ananassa* Duch.) cultivars (Camarosa, Cristal, Monterey and Portola). The four cultivars showed an increasein their red coloration during fruit development. The anthocyanin accumulation in the four cultivars was related to the particular progress of the transcriptional activity of genes involved in the biosynthesis of flavonoid pigments. The greatest increase was observed in 'Monterey' and 'Camarosa', thus we have found a correlation between fruit color redness (*a**) and total anthocyanins only in these two cultivars. However, a positive correlation between the mRNA abundance of *FaF3'H* and *FaFLS* and the total flavonoids content was found in the four cultivars. Finally, we found correlations between color and other important physiological properties as firmness and aroma expressed as total esters. These results could be useful in making decisions in future breeding programs to improve the content of healthy compound content in strawberry fruits.

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Comparative study of several molecular markers expressed in gills of Atlantic salmon through real-time PCR

Morera Francisco J. [1], Marcos Castro[1], Daniela Nualart[2], Loncoman Carlos A[1], Jose Luis Muñoz[3], Luis Vargas-Chacoff[2,4].
(1) Farmacologia y Morfofisiologia, Ciencias Veterinarias, Universidad Austral de Chile, Valdivia, CL
(2) Ciencias Marinas y Limnologicas, Ciencias , Universidad Austral de Chile, Valdivia, CL
(3) Centro I-MAR, Universidad de Los Lagos, Puerto Montt, CL
(4) Centro FONDAP de Investigacion en Altas Latitudes (IDEAL), Ciencias , Universidad Austral de Chile, Valdivia, CL

In the salmon industry, smoltification is a crucial point in the productive chain because smolt quality has a direct impact on production efficiency. Indeed, one of the largest economic losses in Chilean salmon aquaculture are the juvenile salmon seeded in seawater that are unable to acclimate to their new environment either owing to an incomplete smoltification process (called “delayed or unadapted”). Any significant improvement in determining with more accuracy the smoltification and defining the optimal transition time point of smolts from freshwater to seawater will have a major impact in the productivity of salmon aquaculture. At the research laboratory level, the expression of key molecules that form the salt secretion machinery in gills of salmonids correlate, to some degree, with seawater adaptation. Nonetheless, the Chilean salmon industry has not yet to adopt these potential markers probably due to the lack of quantitative data demonstrating a significant improvement over Na+/K+ATPase (NKA) measurements alone. Here, we evaluate levels of other molecular markers, in addition to NKA activity to estimate the optimal timing of smolt transfer to seawater during salmon production. The main goal is to determine the predictive value of different molecular markers expressed during smoltification of Atlantic salmon as indicators ofsmolt survival and performance in seawater,and make this information available to the scientific community and salmon farmers.
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Using virus-like particles of hantaviruses as a platform for the induction of protective immune responses

Nicolás Muena[1], Eduardo Bignon[1], Pablo Guardado-Calvo[2], Felix Rey[2], Nicole Tischler[1].
(1) Laboratorio de Virología Molecular, Fundacion Ciencia y Vida, Santiago, CL
(2) Departement de Virologie, Institut Pasteur, Paris, FR

Hantaviruses are rodent-borne zoonotic pathogens and in America they produce hantavirus pulmonary syndrome (HPS) with high mortality rates. In South America, most human cases are produced by Andes virus (ANDV).To date, there are no preventive or therapeutic treatments approved by the FDA. Hantaviruses infect pulmonary endothelial cells, a process mediated by the envelope glycoproteins of the virus, Gn and Gc which are responsible for binding to cellular receptors and Gc drives virus-cell membrane fusion after the viral uptake into acidic endosomal compartments. The aim of the present work is to induce neutralizing antibody responses against ANDV by the use of non-infectious virus-like particles (VLPs). Using VLPs, we have developed monoclonal antibodies (mAbs) that bind Gn and Gc glycoproteins and show cross-reactivity against glycoproteins of other hantaviruses. One mAb showed partial neutralization against ANDV and its binding site was determined in Gc by peptide scanning, suggesting that it could inhibit the fusion step. This was confirmed by fusion assays *in vitro* observing a partial inhibition of this process. The partial inhibition of the virus is concordant with our recent finding that the viral surface is highly dynamic fluctuating between open and closed conformation; among which the latter is not compatible with infection. Considering this, we introduced several modifications to the VLPs production to enhance spike stability in its closed conformation. *In vitro* infection assays showed higher neutralizing activity of polyclonal sera obtained with these VLPs compared to wt VLPs, providing valuable information for the design of protective immune responses.

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A proteogenomics approach to construct a reference proteome for saliva in Chilean women

Guillermo Nourdin[1], Mauricio Hernandez[1], Francisco Alvarez[1], Pablo Saldivia[1], Alan Fritz[1], Cristian Vargas[2], Elard Koch[1].
(1) Division of Proteogenomics, MELISA Institute, San Pedro de la Paz, CL
(2) Clinical Protocols, Division of Epidemiology, MELISA Institute, San Pedro de la Paz, CL

Proteogenomics are defined as the combination between proteomics and genomics techniques, which allows the construction of databases for specific populations in order to improve the identification of peptides not found in canonical sequences with conventional nLC-MS/MS.

We use a protegenomics approach to investigate the proteome of human saliva samples (n=6) from healthy young woman at fertile age. Transcriptome libraries were obtained through TruSeqHT kit and sequenced in house using NextSeq 500 platform (Illumina). The results were filtered by quality, adapter cutting, filter by species and assembly. The result was translated with GeneMark-HMM software and was used as reference for subsequent proteomics analysis. Proteins were extracted utilizing a standard protocol. The obtained mix of peptides were quantified by IR and solved by nanoHPLC (nanoElute) Mass Spectometry using our TimsTOFpro instrument (Bruker). Protein identification was performed using the PEAKS studio X with the transcriptomic database generated above. The results were annotated against Uniprot/Swisprot database (560,292 entries). Functional annotation was performed through KAAS, GO and Reactome.

Overall 23.754 human transcript were obtained representing an average of 11,2% of total mRNA in samples. These sequences were used as customized database and combined with the MS results, which produced a total of 36,243Peptide-Spectrum Matches. A total of 2,513 peptides and 668 proteins were identified by this combined approach. Functional annotation identified proteins know to be constitutive from saliva, sugar metabolism and dental apparatus.

Proteogenomics appear to be a promissory approach for the research of potential biomarkers of human health and disease in Chilean women.

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Root morphogenic responses under salt stress: contribution of auxin perception and signaling

Jose Antonio OBrien[1].
(1) Department of Molecular Genetics & Microbiology/Department of Fruit Production and Enology, School of Biological Sciences/School of Agricultural and Forest Sciences, Pontificia Universidad Católica De Chile

High salt concentration in soil modulates in a negative manner plant growth and development due to an increase in osmotic stress and ion toxicity. In roots, high soil salinity causes inhibition of both primary root elongation and number of lateral roots. Auxin has a key role in the modulation of root architecture throughout the life cycle and in response to environmental stresses. To achieve this, auxin has a complex signaling pathway that involves the perception of the hormone, degradation of a repressor complex and the transcriptional expression of auxin-responsive genes. Auxin F-box 3 receptor (AFB3) is one of the four auxin receptors described in Arabidopsis thaliana. It has been found that this receptor is involved in the modulation of root architecture, depending on the availability of nutrients in the soil. Here we showed that over-expression of AFB3 exhibited increased resistance to salt stress in terms of lateral root density and germination rate. We also studied the downstream signaling components to further characterize the role of auxin in response to salt stress. Additionally, we have studied a number of transcription factor involved in the modulation of auxin signaling and transport in response to salt stress. These results give lights of the possible mechanism that leads to the modulation of the root system architecture in response to salt stress. The effect on root plasticity commanded by the auxin signaling pathway could then be modulated to give a tolerant phenotype under stress conditions allowing better performance in unfavorable environments.

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Cell wall metabolism in contrasting firmness table grapes during development and ripening

Patricio Olmedo[1], Cristóbal Balladares[1], Pablo Sepúlveda[1], Joaquín Delgado-Rioseco[1], Baltasar Zepeda[1], Francisca Blanco-Herrera[1], Claudio Meneses[1], Defilippi Bruno G[2], Romina Pedreschi[3], Reinaldo Campos-Vargas[1].
(1) Centro de Biotecnología Vegetal, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago, CL
(2) Unidad de Postcosecha, INIA La Platina, Instituto de Investigaciones Agropecuarias, Santiago, CL
(3) Laboratorio de Fisiología Postcosecha y Bioquímica de Alimentos, Facultad de Ciencias Agronómicas y de los Alimentos, Pontificia Universidad Católica de Valparaíso, Quillota, CL

Fresh fruit exportation is one of the main sources for incomes in Chile, and table grape is the most cultivated species. Once harvested, grape clusters must reach their final markets, resulting in long cold storage events. Therefore, the quality parameters of the grape bunches decrease and the firmness of the berries is one of the most affected traits. Firmness is a parameter that oscillates throughout the development of the grape berry, showing a lower firmness at the harvest than at the immature stages. In the present work, we evaluated berries of *Vitis vinifera* (L.) cv. Thompson Seedless displaying contrasting firmness at four stages of development and ripening and we studied the transcriptomic and metabolomic profiling and cell wall components. Our results revealed that at each stage of development several cell wall related genes are differentially expressed. The softer phenotype showed an increase in the expression of genes mainly involved in xyloglucan and pectin degradation. Additionally, the berries obtained from the firmer phenotype contained more soluble galactinol and fructose-6-phospahate at veraison than the softer phenotype based on metabolomic analyses. Moreover, the softer phenotype accumulated more UDP-glucuronic acid, glucose and ribose at harvest. The cell wall monosaccharide composition analysis by HPAEC-PAD indicated significant differences between sugars related to rhamnogalacturonan-I, homogalacturonan and xyloglucan structures. These results suggest a differential cell wall metabolism in both hard and soft phenotypes and could be influencing texture dynamics. Thus, this work attempts to understand the link between cell wall dynamics and berry firmness during development and ripening.

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Ethnicity-driven T-cell Epitope Prediction for Rational Vaccine Design

Patricio Oyarzún[1], Victor Fica[1], Manju Kashyap[1], Alexis Salas[2], Bostjan Kobe[3].
(1) Laboratorio de Nanotecnología, Facultad de Ingeniería y Tecnología, Universidad San Seabstián, Concepción, CL
(2) Departamento de Farmacología, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, CL
(3) Institute for Molecular Bioscience, Faculty of Science, The University of Queensland, Brisbane, AU

There is a strong demand for next-generation vaccination approaches improving T cell-mediated immunity. A promising approach is to focus vaccine development on the antigenic determinant regions that are specifically recognized by T-cells (T-cell epitopes). T-cell epitopes are peptides presented by major histocompatibility complex (HLA in humans) proteins, leading to T-cell activation and stimulation of cellular immune responses (epitope-based approach). However, in the context of genetically heterogeneous human populations, HLA alleles are expressed at different frequencies and individuals displaying a different set of alleles are likely to react to regions from a given pathogen. The immunoinformatics method Predivac builts its prediction on specificity determining residues (SDRs), a small set of amino acids at the peptide:protein binding interface. Given a pathogen proteome and a target population (geographic region), the program uses HLA allele frequency data to calculate and optimize the fraction of individuals potentially covered by the epitopes. The performance for HLA class I and II binding prediction was determined by leave-one-out cross-validation, while epitope prediction was determined with a dataset of experimentally validated T-cell epitopes. Predivac-2.0 was written in Python and validated for CD4+ and CD8+ T-cell epitope prediction and population-based selection. The overall accuracy (AUC) of Predivac on CD4+ and CD8+ T-cell epitopes, was AUC=0.71 and AUC=0.84. Finally, Predivac-2.0 was capable of identifying a number of experimentally determined immunodominant T-cell epitopes reported in HIV (Thailand population). We demonstrated that Predivac-2.0 was strongly capable of identifying both CD8+ and CD4+ T-cell epitopes, as well as immunodominant sequences of HIV proteins.

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Addressing the role of *T. atroviride*hypothetical circadian clock components in the tripartite fungal-plant-fungal interaction with *A. thaliana*and *B. cinerea*

Rodrigo Pérez-Lara[1,2], Aldo Seguel [1,2], Marlene Henriquez[1,2], Larrondo Luis F.[1,2].
(1) Departamento de Genética Molecular y Microbiología , Facultad de Ciencias biologicas, Pontificia Universidad Católica De Chile, Santiago, CL
(2) Millennium Institute for Integrative Biology (iBio), CL

Fungi from the Trichoderma genus are known for their biocontroller ability, which made them valuable agents to protect plants against pathogens. In particular, when *Trichoderma atroviride* interacts with *Arabidopsis thaliana*, it can prime plant defenses increasing gene expression from both SA and JA/ET pathways, which improves the tolerance against biotrophic and necrotrophic phytopathogens. The underlying mechanism by which *T. atroviride* modulates these effects is unknown. However, it has been described that the fungal circadian clock in the interaction between *A. thaliana* and the phytopathogen *Botrytis cinerea* is important. Although no functional clock has been reported for *T. atroviride,* it possesses homologs of the blue light receptor WC-1 (BLR-1) and the central oscillator FREQUENCY (Tafrq), which have been extensively described for the circadian clock of *Neurospora crassa*. To determine if the circadian clock from *T. atroviride* is involved in the outcome of the interaction between plants and Trichoderma, we treated Arabidopsis plants with *T. atroviride* WT and mutant spores for *blr1* and *frq* (ΔBLR1, ΔTafrq) to evaluate growth promotion. Also, we inoculated Trichoderma treated-plants with *B. cinerea* to assess plant primed defense when BLR1 and FRQ are absent in Trichoderma. Besides, we analyzed PR-1 protein levels as an indicator of systemic acquired defense in the plant. We have observed differential growth promotion in ΔBLR1 treated-plants, suggesting a role for the components of the putative Trichoderma circadian clock in the interaction. This line of work will help unveiling the role of clock components, and environmental conditions, in the interplay of organismal interactions
Millennium Institute for Integrative Biology (iBio), FONDECYT 1171151.

Seed and seedling chemical and biological interactions among four tree species from Mediterranean forests, in post-fire context

Plaza Valencia Âlvaro J. [1,2], Miguel Castillo[1], Naulin Paulette I.[2].
(1) Laboratorio de Incendios Forestales, Departamento de Gestión Forestal y su Medio Ambiente, Facultad de Ciencias Forestales y de la Conservación de la Naturaleza, Universidad de Chile, Santiago, CL
(2) Laboratorio Biología de Plantas, Departamento de Silvicultura y Conservación de la Naturaleza, Facultad de Ciencias Forestales y de la Conservación de la Naturaleza, Universidad de Chile, Santiago, CL

Around the world, there are five Mediterranean-Climate Regions. One of them corresponds to Central Chile. Like other Mediterranean regions, this area is characterized by its high biodiversity and its high anthropogenic degradation, mainly due to wildfires. In order to generate new strategies for the restoration of the biomes of this area, a combination of native tree species was selected, based on two criteria: 1) their natural presence in Chilean Mediterranean forest and 2) the speed of germination. The species chosen were *Acacia caven*, *Caesalpinia spinosa*, *Prosopis chilensis* and *Quillaja saponaria*. We analyze how the seeds of each of these species responded to the presence of raw chemical extracts obtained from seeds of the other species, differentiating the effect of these other seeds when they were dormant (by using tegument and testa extracts), from when they were germinated (through extracts of cotyledons and embryos), finding many allelopathies, auto-allelopathies and facilitations. We also analyzed the survivorship and growth in post-fire conditions of each of these species in two situations, 1) when they were growing in monospecific condition (control) or 2) in combination with the other species (treatments). It was found that many of the interactions observed between the seeds were perpetuated during the seedling stage, even in those cases in which the effector individual had died. In conclusion, we demonstrate that non-established individuals of a natural community are able to actively interact with other organisms. This is especially important when proposing combinations of species for reforestation or restoration of degraded ecosystems.

CONAF project 010/2019 “Determinación de las interacciones biológicas y químicas intra e interespecíficas para mejorar el hábitat de *Prosopis chilensis* en el Bosque Espinoso”

Phospho-mimetic ECE1c^{T9D} promotes *in vitro* drug resistance, migration and colony-formation of colorectal cancer cells

Camila Quezada[1], Natalia Landeros[1], Pablo Pérez[1], Julio Tapia[1].
(1) Departamento de Oncología Básico Clínica, Facultad de Medicina, Universidad de Chile, santiago, CL

Introduction:
Endothelin-converting enzyme-1c (ECE1c) is a membrane metalloprotease responsible for the activation of the mitogenic peptide Endothelin-1. Our previous results suggest that phosphorylation of ECE-1c in residues Thr-9, Ser-18 and Ser-20 by the protein kinase CK2, promotes stability by preventing its proteasomal degradation, also increasing the invasiveness of DLD-1 CRC cells. Although only phosphorylation of Ser-18 and Ser-20 was confirmed by MS, results obtained by using triphospho-mimetic and super-stable mutants lead us to suspect that Thr-9 may be involved in its stability and down-strain cellular effects.

Methodology:
DLD-1 clones expressing FLAG-tagged ECE-1c wild-type, phospho-mimetic (ECE1c^{T9D}) or phospho-resistant (ECE1c^{T9A}) mutants were used. Protein stability of all ECE1c forms was performed by using the translation inhibitor CHX. Drug resistance was evaluated by MTS assay in presence of 5-FU and/or CK2 inhibitor CX-4945. Protein expression of survivin was measured by western blot. Wound-healing and *soft-agar* assays were performed for evaluating *in vitro* migration and colony-formation, respectively.

Results:
The change of Thr-9 to a phospho-mimetic residue of Asp led to higher stability of ECE1c even in presence of CX-4945. ECE1c^{T9D} promoted resistance to 5-FU and CX-4945, which correlated with an increase of survivin levels. ECE1c^{T9D} also promoted migratory and clonogenic capacities of DLD-1 cells.

Conclusions:
High stability promoted by phosphorylation of ECE1c at Thr-9, which is putatively achieved by CK2, confers malignant traits to CRC cells that are associated with tumor-growth and metastasis. Findings of this work are a first step in the study of the mechanism by which ECE1c may promote aggressiveness in CRC patients.

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Unveiling the sequence and structure features enabling enzymatic degradation of PET at low temperatures

Paula Blazquez[1], Pablo Galaz-Davison[1], Felipe Engelberger[1], Victoria Guixé[2], Marcos Sotomayor[3], Wolfgang Zimmermann[4], **Ramirez-Sarmiento Cesar A [1]**.
(1) Institute for Biological and Medical Engineering, Schools of Engineering, Medicine and Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, CL
(2) Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, CL
(3) Department of Chemistry and Biochemistry, The Ohio State University, Columbus, OH, US
(4) Institute of Biochemistry, Department of Microbiology and Bioprocess Technology, University of Leipzig, Leipzig, DE

Polyethylene terephthalate (PET) is a highly consumed plastic with an annual production of 50 million tons, but its low biodegradability leads to its high accumulation as waste. However, several hydrolases with PET degradation activity have been found in thermophilic fungi and bacteria, whose optimal activities at high temperatures are due to PET becoming more accessible for enzymatic degradation. Strikingly, it has been recently described that a PET hydrolase from Ideonella sakaiensis (termed PETase) degrades PET at room temperature. This enzyme could be cornerstone for the efficient treatment of PET waste worldwide, wherefore is crucial to understand the molecular basis of its activity at lower temperatures. Here, we determined the crystal structure of PETase at 2.02 Å. Then, we observed via molecular dynamics that the active site of PETase has higher flexibility at room temperature than thermophilic hydrolases. This flexibility is controlled by a novel disulfide bond in its active site, which stabilizes its catalytic triad and is experimentally relevant for enzyme activity. Lastly, molecular docking of a model substrate predicts that PET binds in a unique conformation due to key substitutions within its active site, whose energetic contributions to PET binding have been experimentally validated. Reasoning that other enzymes could also degrade PET at room temperature, we identified and biochemically characterized two PET hydrolases from Antarctic organisms. These enzymes exhibit high degradation activity against polycaprolactone and potential degradation of PET films at room temperature. Our results are valuable for rationally increasing the PET degradation efficiency of PETase and similar enzymes.

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Analysis of ligands for Mn^{2+} in agmatinase like protein (ALP)

Maria-Belen Reyes[1], Kelly Mella[1], Martínez Ignacio[1], Carolina Alarcon[1], José Martínez-Oyanedel[1], Elena-Amparo Uribe[1].
(1) Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas , Universidad de Concepción, Concepción, CL

Agmatine, a precursor for polyamine biosynthesis, is also associated with neurotransmitter, anticonvulsant, antineurotoxic and antidepressant actions in the brain. Recently, we have described a new protein that hydrolyzes agmatine, the *agmatinase-like protein* (ALP), it was identified in rat brain. ALP, in spite of differing in amino acid sequence, it is strictly dependent on Mn^{2+} for its catalytic activity. However, the Mn^{2+} ligands are yet undefined and any approximation to the enzyme active site is impeded by the lack of structural information. We have generated a comparative structural model, considering the very low sequence similarity (30-38%) between ALP and the crystal structures of prokaryotic agmatinases, and we have proposed new ligands for the Mn^{2+} cofactor. A simple mutant D215A, a double mutant E287A/K289A and a triple mutant N211A/Q213A/D215A of these putatives Mn^{2+} ligands in ALP, not presented agmatinase activity. In addition, we cloned and expressed a sequence of 210 amino acids (central-ALP), that include the putative metal-ligands (ALP contain 523 aa). The results indicated that central-ALP is catalytically active, with a *Km* for agmatine of 1,2 ± 0,37 mM similar to wild type, but with a decreased catalytic activity. Central-ALP resulted to be activated by Mn^{2+} with a activation constant of $2{,}18 \times 10^{-8}$ M for Mn^{2+}, like to wild-type ALP. These results, indicate that ALP-central contain the active site for agmatine hydrolysis and together with of mutants studies, support our proposal of new Mn^{2+} ligands in the active site of ALP.

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The fold-switch of the cyanobacterial metamorphic protein KaiB is uncoupled of its dimerization

Maira Rivera[1], Pablo Galaz-Davison[1], Cesar Ramirez-Sarmiento[1].
(1) Instituto de Ingeniería Biológica y Médica, Facultad de Ingeniería, Ciencias Biológicas y Medicina, Pontificia Universidad Católica de Chile, Santiago, CL

Metamorphic proteins exhibit dramatic structural changes that are often involved in crucial biological processes, such as the synchrony of cyanobacterial physiology with day/night cycles. This process is controlled by the simplest circadian clock in nature: a molecular clock composed by three proteins (KaiABC) that establish a phosphorylation oscillator. The clock is regulated by a fold-switch of the metamorphic protein KaiB. This protein goes from a diurnal tetrameric structure (gsKaiB) to a thioredoxin-like fold (fsKaiB) that binds to KaiC, allowing the entrance to nocturnal physiology. Despite the relevance of the structural metamorphosis of KaiB, little is known about the molecular details of its transformation. Here we studied the folding mechanism and the local differential stability between the two folds of KaiB *in silico*. Molecular dynamics simulations using coarse-grained structure-based models showed that the monomer of gsKaiB and fsKaiB have a two-state folding mechanism with similar activation energies. However, the dimeric form of gsKaiB has two folding routes, being the more favorable the one in which each monomer folds separately before dimer association. To determine the local stability and per-residue contributions to the structural transformation of KaiB, we performed confine-convert-release molecular dynamics. Our results show well-defined regions that differentially stabilize gsKaiB or fsKaiB, particularly within metamorphic region. These results are in good agreement with limited experimental information available for both native states. Altogether our results suggest that gsKaiB dissociates prior to perform any structural change and that this process is facilitated by flexible regions in the KaiB structure.

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Spatiotemporal analysis of the root nitrate response identifies key factors commanding nitrate-regulatory networks in the endodermis cell-layer

Eleodoro Riveras[1,2,3], Orlando Contreras-López[1,2,3], Vidal Elena A.[3,4], Moyano Tomas C.[1,2,3], Alvarez José M.[1,2,3], Gutiérrez Rodrigo A.[1,2,3].
(1) Departamento de genética molecular y microbiología, Facultad de ciencias biológicas, Pontificia Universidad Católica de Chile, Santiago, CL
(2) FONDAP Center for Genome Regulation, CL
(3) Millennium Institute for Integrative Biology , CL
(4) Centro de Genómica y Bioinformática, Facultad de ciencias, Universidad Mayor, Santiago, CL

Nitrate is a potent nutrient signal that can impact global gene expression in plants. To understand the spatiotemporal dynamics of the root nitrate response and to identify key nitrate regulatory factors, we performed gene expression profiles in five major cell types of the root over a time-course after nitrate treatment. All cell-types showed regulation of gene expression at early time points, indicating rapid organ-wide response to nitrate. Known biological processes showed a spatiotemporal pattern of regulation from epidermis to inner layers of the root. Consistent with a key physiological role in communicating with the environment, endodermis showed the highest number of differentially expressed genes in response to nitrate treatments, and most complex regulatory networks as compared to other cell-types. Using integrative regulatory network analysis and experimental validation we identified new transcription factors that mediate nitrate responses in root organs. TARGET, ChIP-Seq and Yeast-1-Hybrid analysis validated these new transcription factors are able to control the expression of a large fraction of endodermis nitrate-responsive genes with functions in nitrate uptake and assimilation, by direct binding or by transcriptional control of TGA1 and TGA4 transcription factors. Phenotypic analysis of single and double mutants for the new regulatory factors showed altered lateral root density in response to nitrate. Our results provide a spatiotemporal view of nitrate responses in roots that provide insights into the complex orchestration of the root response to an important nutrient environmental signal and identify novel regulatory players in the nitrate response of Arabidopsis.

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Studying the interaction between the signal peptide for reticular translocation and the Sec61 translocon with force spectroscopy

Luka Robeson[1], Nathalie Casanova-Morales[1], Francesca Burgos-Bravo[1], Carolina Ramírez[1], Wilson Christian A.M.[1].
(1) Laboratorio de Bioquímica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, CL

The protein-conducting channel Sec61 found in the membrane of the endoplasmic reticulum allows the translocation of proteins from the cytosol to the reticular lumen and insertion into the ER membrane, decisive steps in the biosynthesis of most extracellular and transmembrane proteins. These secretory proteins possess a “signal peptide” (SP) at their N-terminus, which interacts with the translocon and begins translocation. Mutations in the signal peptide can preclude translocation and cause diseases related to the intracellular accumulation of these proteins, indicating an essential role for this signal peptide-Sec61 interaction; however, the binding parameters that characterize these interactions remain to be defined. Single molecule force spectroscopy using optical tweezers was used to measure the adhesion frequency and the forces necessary to dissociate the interaction between Sec61 and the SP of Prepro-alpha-factor (PpαF), WT and a translocation-deficient mutant (Ala13Glu). Dudko-Hummer-Szabo models were applied to the rupture forces histograms to calculate the mean lifetime of interaction in dependence of the force [τ(F)]. From the latter, the lifetime and the distance of the transition state of the dissociation process at zero force were obtained (τ_0=5±1 s and $\Delta x^{\ddagger}0$=0.06±0.02 nm for WT; τ_0=4±1 s and $\Delta x^{\ddagger}0$=0.04±0.02 nm for Ala13Glu, respectively). Also, WT SP interacts significantly more frequently (~49%) with the translocon than the mutant (~31%). This suggests that the translocation deficiency observed with Ala13Glu mutant could be related to a slower association rate between the SP and the Sec61 translocon instead of an increase in the dissociation constant.
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A Comparative Study on the Antioxidant Activities and Phenolic Contents of Extracts obtained from Chilean Endemic Plants

Carlos Schneider[1], Carlos Anabalón[1], Lizeth Gallegos[1], Natali Salcedo[1], Lorena Tapia[1], Daniel Troncoso[1], Luis Valenzuela[1], Katerine Vega[1].
(1) Departamento de Ciencias y Tecnología Vegetal, Escuela de Ciencias y Tecnologías, Universidad de Concepción, Los Angeles, CL

1Universidad de Concepción, Campus Los Angeles, Departamento de Ciencias y Tecnología Vegetal, Escuela de Ciencias y Tecnologías, J.A. Coloma 0201, Los Angeles, Chile. cschneider@udec.cl Plants have evolved protective mechanisms to keep deleterious reactions to a minimum *via* antioxidative defence. These reactions are caused by reactive oxygen species (ROS) and UV radiation increased the production of ROS. Plant polyphenols are secondary metabolites that widely exists in plants, and these metabolites are natural antioxidants. In this investigation, an antioxidant effect in methanolic extracts (ME) and aqueous exctracts (AE) of *Acaena pinnatifida*, *Eucryphia glutinosa*, *Kageneckia oblonga* and *Rhaphitamnus spinosus* was assayed with 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical and 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) cationic radical. Results were expressed in gallic acid and trolox equivalents for DPPH and ABTS assays, respectively. In the DPPH and ABTS assays, a inhibitory concentration of extract needed to inhibit 50% of the absorbance (IC50 value) was calculated. The concentration of total polyphenols in aqueous and methanolic extracts , was determined according to the Folin-Ciocalteu method. The results of IC50 values measured by the DPPH method were: 0,159 mg/mL (ME from roots of *Acaena pinnatifida*), 0,568 mg/mL (ME from aerial parts of *Acaena pinnatifida*),0,378 mg/mL (AE from branches of *Eucryphia glutinosa*), 0,427 mg/mL (AE from leaves of *Eucryphia glutinosa*), 0,308 mg/mL (AE from leaves of *Kageneckia oblonga*), 0,336 mg/mL (ME from leaves of *Kageneckia oblonga*), 0,560 mg/mL (ME from leaves and stems of *Rhaphitamnus spinosus*), 3,655 mg/mL (ME from fruits of *Rhaphitamnus spinosus*).

FREQUENCY, a canonical circadian protein with novel roles in the plant- pathogenic fungus *Botrytis cinerea*

Aldo Seguel[1], Javier Canales[2], Müller Hanna[1], Luis Larrondo[1].
(1) Genética Molecular y Microbilogía, Ciencias Biológicas, Pontificia Universidad Católica De Chile
(2) Instituto de Bioquímica y Microbiología, Ciencias, Universidad Austral de Chile, Valdivia, CL

The fungus *Botrytis cinerea*is a relevant necrotrophic phytopathogen from both an economical and scientific perspective. A key elementinBotrytis virulence is light, which isable to regulate its development and behavior. Botrytis possesses a circadian clock constituted by the negative regulator frequency (BcFRQ1) and a heterocomplex, that regulates BcFRQ1 expression, composed by White Collar 1 and White Collar 2 (BcWCL1, BcWCL2). When BcFRQ1 is deleted, the *Δbcfrq1* mutant strain turns into an "always sclerotia" phenotype. On the other hand, neither the *Δbwcl1* mutant nor any other FRQ mutants in filamentous fungi have shown this phenotype. The *Δbcfrq1*phenotype can be reverted by adding primary sources of nitrogen to the media. This evidence suggests that BcFRQ1 hasextra-circadian roles in *B. cinerea*. In order to determine which metabolic pathways are disturbed in the absence of BcFRQ1, an RNAseq was performed on *Δbcfrq1*strains. Mutant and wild type strains were cultured with or without glutamine, under two different light conditions: constant light (LL), and constant darkness (DD). We observed changes in the expression of 1090 genes when comparing the *Δbcfrq1*strain with the WT (568 genes upregulated, 522 genes downregulated). Most of these genes are related to the mitochondrial function, which has been suggested to be a link between circadian clock and metabolism. These results demonstrate that nutrition assimilation pathways are severely affected in the *Δbcfrq1*mutant. This work can help to understand the evolutionary origins and specialization of FRQ in clock and metabolic crossroads.

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The shape of gold nanoparticles alters the aggregation kinetic of amyloid-b peptide

Andreas Leonardo Tapia[1,2,3,4], Eduardo Gallardo-Toledo[2,1], Rodrigo Rivera[3], Natàlia Carulla[4], Mauricio Baez[3], Marcelo Kogan[2,1].
(1) Departamento de Química Farmacológica y Toxicológica, Ciencias Químicas y Farmacéuticas, Universidad de Chile, Independencia, CL
(2) Centro Avanzado de Enfermedades Crónicas, ACCDiS, CL
(3) Departamento Bioquímica y Biología Molecular, Ciencias Químicas y Farmacéuticas, Universidad de Chile, Independencia, CL
(4) CBMN (UMR 5248), University of Bordeaux-CNRS-IPB, Institut Européen de Chimie et Biologie, FR

The nanotechnology has gained a great relevance in the nanomedicine area, where the gold nanoparticles (GNP) have been highlighted because of their simple synthesis, biocompatibility and they possess a big surface area to be functionalized with different molecules (drugs, peptides, etc). For these reasons, GNP are being used like strategy to treat neurodegenerative diseases, as in the aggregation process of amyloid-bpeptide (Ab) related to Alzheimer´s disease. However, how the GNP shape affect the Abaggregation process is poorly studied. To address this problem, we studied the effect of different shapes of GNP on Abaggregation process by performing global fitting of several models to describe the aggregations kinetics obtained at different protein concentrations. In absence of GNP, the molecular mechanism of Abaggregation has been described by a secondary nucleation multi-step model, but in the presence of a flat surface of gold nanoprisms (GNPr), the aggregation process is accelerated. Specifically, the rate constant associated with the steps of elongation and secondary nucleation increases in two orders of magnitude. In another hand, in the presence of a curve surface of gold nanospheres (GNS), this constant decreases in one order of magnitude and the molecular mechanism of aggregation changes from secondary nucleation to fragmentation/ secondary nucleation. We hypothesize the GNPr surface works like a scaffold that increases the local concentration and promotes the secondary nucleation, accelerating the Abaggregation process. In another hand, a curved surface forces the aggregates to interact with the GNS promoting the fragmentation of the aggregates.

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Bioprospection of bacteria associated with halophytic plants enhancing salt tolerance in *Arabidopsis thaliana*

Ricardo Tejos[1], Tamara Godoy[1], Rodrigo Gonzalez[1], Damaris Reyes[1].
(1) Laboratorio de Biotecnología Celular, Facultad de Recursos Naturales Renovables, Universidad Arturo Prat, Iquique, CL

Plants need to appropriately sense and respond to all surrounding abiotic factors in order to regulate their physiology and development in an appropriate manner. Acute changes in ambient temperature, soil condition, and nutrient and water availability impact on how plants grow and reproduce, and eventually deteriorate plants productivity. On the field, plants are exposed to several abiotic environmental factors and major economic losses arising from salt, drought, heat, and water-deficit stresses. Plants deploy a diverse set of signaling molecules to regulate stress responses, and increasing evidence demonstrates the important role their associated microbiota play in modulating such response. We collect bacteria associated to the rhizosphere of halophytic plants living in environments with highly saline soils (salt flats and river mouths located in north Chile) and perform a screen to find beneficial bacteria and their metabolites using the model plant *Arabidopsis thaliana* as the bio-prospecting tool. We have generated a collection of rhizobacteria and co-cultivated them *in vitro* with *Arabidopsis* seedlings in order to select those improving plant salt tolerance. From the initial collection, we selected the ten most promising candidate rhizobacteria and extracted soluble (not volatile) secreted rhizobacteria metabolites and further tested them as the candidate factors for improving *Arabidopsis* salt tolerance. We are currently characterizing the effect rhizobacteria and the metabolites on *Arabidopsis* developmental response and hormonal pathways involved in salt tolerance and other abiotic stresses such as heat and osmotic stress.

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Interaction of HTLV-1 retroviral protein Tax with host proteins such as calreticulin in infected lymphocytes of patients with spastic paraparesis

Valenzuela Marìa A [1], Sebastián Quintremil[1], Carolina Alberti[1], Fernando Medina[1], Javier Puente[1], San Martin Maria E[1].
(1) Departamento de Bioquimica y Biologia Molecular, Ciencias Químicas y Farmacéuticas, Universidad De Chile, Santiago, CL

Introduction: HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is due to HTLV-1 infection of $CD4^+$-T-lymphocytes characterized by a progressive corticospinal tract axonopathy. The extracellular action of the viral protein Tax is involved in this neurodegeneration. It is known that the viral protein $p12^I$, required for lymphocyte infection, interacts with calreticulin (CRT), localized in the ER and cis-Golgi apparatus. The aims of this research include to follow: the secretion of Tax and CRT from patient PBMCs (peripheral-blood mononuclear cells) and the extracellular interaction of Tax with host proteins to understand the neurodegenerative action. Methods: 1) PBMCs of HAM/TSP patients were incubated with inhibitor and activators of transport ER to the Golgi, Brefeldin and ionomycin plus phorbol myristate. 2) PC12 cells during NGF neuronal differentiation were incubated with supernatants of HTLV-1-infected MT-2 cells. Interaction between proteins were determined by co-immunoprecipitation and pull-down and then followed by western-blot. Results: The results agree with a canonical secretion mechanism for Tax and CRT in HTLV-1-infected PBMCs. Tax and two host proteins, CRT and Sema4D were found increased in MT-2 culture-medium an HTLV-1 infected cell-line compared with non-infected lymphocytes. Western-blot of co-immunoprecipitated Tax, CRT and Sema4D showed that Tax interacts with both proteins. Reduction of PC12 neurite elongation by culture-medium of MT-2 is block only by antibodies against Tax and Sema4D. Conclusions: Tax and CRT co-secreted from infected PBMCs, and PC12 results showed that CRT is not responsible of the neurodegenerative effect of Tax, instead the complex of Tax-Sema4D mediated by Plexin signal transduction could be involved.

Unraveling the AMP allosteric regulation mechanism of bifunctional ADP-dependent sugar kinases from archaea

Gabriel Vallejos[1], Herrera Sixto M[1], Kaufman Sergio B[2], González-Lebrero Rodolfo M[2], Víctor Castro-Fernandez[1], Victoria Guixé[1].
(1) Departamento de Biología, Facultad de Ciencias, Universidad de Chile
(2) Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, AR

In several archaea the Embden–Meyerhof pathway presents unique modifications, such as the ADP-dependence of glucokinase (GK) and phosphofructokinase (PFK) activities. In organisms from the order *Methanococcales* one bifunctional enzyme performs both activities. All these enzymes have been reported as non-regulated. Surprisingly, in the bifunctional enzyme from *Methanococcus maripaludis* (MmPFK/GK) it was found that both activities are activated by AMP, its reaction product. Here we determine the AMP activation mechanism for the GK and PFK activities, through steady-state kinetics. For both activities, the reaction takes place through an ordered sequential mechanism where MgADP is the first substrate to bind to the enzyme and AMP the last product to be released. Moreover, both activities present inhibition by the sugar substrate, which occurs through the binding of the sugar to the free enzyme, generating a non-productive complex. Also, AMP increases the substrate-inhibition in both activities. The activation mechanism was determined to occur by binding of AMP to an allosteric site that provokes an increase in the sugar affinity at the active site. This also explains the increased substrate-inhibition observed in the presence of AMP. The AMP activations constants were very similar for both activities. However, the increase in glucose affinity due to AMP binding was at least one order of magnitude higher than the increment observed in the affinity for F6P. These results support a differential effect of the allosteric regulator on both activities and would help to shed light on the metabolism of methanogenic archaea and its regulation.

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Soluble IL-6 receptor inhibits PDGF-BB and IL-6 induced vascular smooth muscle cell migration

Paulina Villar[1], Tomás Hernandez-Díaz[1], Ignacio Norambuena-Soto[1], Fernanda Sanhueza-Olivares[1], Nicole Cancino-Arenas[1], Mario Chiong[1].
(1) Centro Avanzado de Enfermedades Crónicas (ACCDiS), Facultad de Ciencias Químicas y Farmacéuticas, Universidad De Chile, Santiago, CL

Vascular smooth muscle cells (VSMCs) are contractile cells that regulate blood flow and pressure. Normally, VSMCs are differentiated with low migration and high contractile protein expression such as smooth muscle α-actin, SM22 and calponin. In pathological vascular remodeling, VSMCs dedifferentiate, decreasing contractile proteins and increasing proliferation and migration. Stimuli such as PDGF BB and IL-6 induce VSMC migration. On the other hand, physical activity reverses pathological vascular remodeling, promoting a differentiated VSMC phenotype. Physical exercise increases the plasma levels of the soluble receptor of IL 6, sIL-6R. However, the relationship between sIL 6R and the differentiated phenotype of VSMCs is not yet known. The objective is to evaluate the effects of sIL-6R on VSMC migration and contractile protein expression induced by PDGF-BB and IL-6. Rat aortic VSMC embryonic cell line, A7r5, was coincubated with sIL-6 and IL-6 or PDGF-BB. Migration was assessed by wound and transwell assays, and by phosphorylation of focal adhesion kinase, FAK. The dedifferentiated state of the VSMC was evaluated by western blotting of α-SMA, SM22 and calponin. PDGF-BB (20 ng/mL) increased VSMC migration. IL-6 (100 ng/mL to 300 ng/mL) increased VMSC migration in a dose-dependent manner. The co-addition of sIL-6R (250 ng/mL), reduced IL-6 (300 ng/mL) and PDGF-BB (20 ng/mL) induced VSMC migration. Moreover, sIL-6R decreased FAK phosphorylation at 60 min. However, no significant changes in contractile protein expression were observed after treatment with sIL6R. Therefore, sIL-6R inhibits VSMC migration induced by PDGF-BB and IL-6. We propose sIL-6R as a potential treatment for pathological vascular remodeling.

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Pilot evaluation of the use of *Bromus sp*, with the application of amendments for the phytostabilization of the tailings, Mina Silva

Aguilar Franchesca Camila [1], Ximena Valenzuela Monsalve[1].
(1) Campus Patagonia, Campus Patagonia, Universidad Austral de Chile, Coyhaique, CL

The purpose of this work is to evaluate the capacity of Bromus sp (Poaceae), together with the organic manure amendment, for the phytostabilization of a Chilean mining tailings, Mina Silva dam, located in Puerto Cristal, with respect to Pb and Zn." For this, a pilot trial with five substrates was implemented: T1 (50% tailings + 25% soil + 25% manure), T2 (25% tailings + 50% soil + 25% manure), T3 (75% tailings + 10 % soil + 15% manure), T4 (100% tailings) and T5 (100% soil). The parameters evaluated with respect to the substrate before and after the test were: CE, pH, percentage of organic matter, N, P, K available, among others. Then the determination of dry biomass of plant tissue of the concentrations of Pb and Zn, both in substrate and in plant tissue, was performed at 60 and 120 days. Preliminary results show the potential of this species in the bioremediation area. However, it is necessary to continue working on new trials that confirm the "tolerance" of the species. By analyzing the results it is possible to establish that the adition of organic amendments to the mine tailings increases the phytostabilization potential of A. nummularia, regarding Zn and Pb, improves substrate conditions, increasing organic matter, N , P, K available. Therefore, the use of this plant species could be recommended, aided by the organic amendment for tailings stabilization.

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Hoffmannseggia doelli: characterization of an extremophile plant from atacama desert

Melissa Aguilar Plaza[1,2], Isabel Pochet[1], Jonathan Maldonado[1], Gabriela Carrasco-Puga[1], Francisca Díaz[1], Claudio Latorre[3], Rodrigo A. Gutiérrez[1].
(1) Genética Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, CL
(2) Agronomía, Ciencias RR. NN y Ciencias Silvoagropecuarias, Universidad Iberoamericana de Ciencias y Tecnología, Santiago, CL
(3) Ecología, Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, CL

Under the current scenario of global climate change, the discovery and characterization of plant species adapted to extreme environmental conditions has become increasingly important. Hoffmannseggia doelli (“mutukuru”) is a perennial herb endemic to the Chilean Atacama Desert that grows between 2900 and 3800 meters above sea level (m a.s.l.) in the western Andes. Its growing habitat is characterized by high radiation (≥620 watts / m2), low water availability (~ 76 mm per year) and rich in toxic mineral soils. Under these conditions, H. doelli can develop a tuberous root, which has served as a food source for the natives of Atacama for centuries. We show that under our experimental conditions H. doelli germinates and grows. We can also get tuberous roots in 45 days. Currently, we have achieved the spread of this species through its tuberous roots. We also performed a nutritional analysis of the H. doelli tuberous root. We compared its composition against native Chiloe´s potatoes, commercial potatoes and yams. We are currently sequencing the genome of H. doelli, in an effort to advance our knowledge about this species and the identification of genes involved in tolerance to extreme abiotic conditions. This work constitutes the first attempt to establish H. doelli cultures under laboratory conditions. We optimize the growth conditions as the basis for future physiological and genetic studies that will help unravel the strategies that allow their growth in extreme abiotic conditions.

Identification of cellular lncRNAs associated to the HIV-1 genomic RNA

Paulina Aguilera-Cortés[1], Cecilia Rojas-Fuentes[1], Daniela Toro-Ascuy[1], Fernando Valiente-Echeverría[1], Ricardo Soto-Rifo[1].
(1) ICBM, Programa de Virología , Facultad de Medicina, Universidad de Chile, Santiago, CL

During its replication cycle, the Human Immunodeficiency Virus type 1 (HIV-1) genomic RNA (gRNA) is assembled into specific ribonucleoprotein complexes (RNPs) whose composition determines its cytoplasmic fate. Several viral and host proteins have been shown to be part of the RNPs of the HIV-1 gRNA, but little is known about regulatory RNAs that are present in these complexes. Despite non-coding RNAs (ncRNAs) particularly long non-coding RNAs (lncRNAs) appear as important post-transcriptional regulators, their role on the late steps of HIV-1 replication has been poorly studied. Indeed, whether lncRNAs are associated to the gRNA of HIV-1 to regulate viral replication is completely unknown. Therefore, the aim of this work was to isolate the RNPs of the HIV-1 gRNA and identify the associated lncRNAs. In order to determine whether lncRNAs are components of viral RNPs, we developed an interactome capture strategy allowing the isolation of the RNPs containing the HIV-1 gRNA from CD4+ T-lymphocytes. Quantification of the HIV-1 gRNA from of the isolated RNPs revealed a 6.000-fold enrichment indicating the selectivity of our interactome capture strategy for the viral genome. We also observed a slight enrichment of the tRNALys3 but not of the ncRNA 7SL in our isolated RNPs. Both RNAs are present in viral particles but only tRNALys3 is directly bound to the gRNA, indicating that our strategy allows the specific purification of the viral genome together with its associated RNAs. We then conducted RNA-seq analyses and succeeded in identifying lncRNAs associated to the HIV-1 gRNA.

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Tolerant INIA tomato rootstock decrease damage in Old Limachino Tomato (OLT) plant during attack of phytopathogen *Pseudomonas syringae pv. Tomato* (*Pst*)

Juan Felipe Alfaro[1], Juan Pablo Martinez[1], Sebastian Molinett[1], Emna Gharbi[2], MingXi Zhou[2], Helene Dailly[2], Michael Seeger[3], Ingrid Ramírez[4], Fernando Dorta[4], Andrea Avila[5], Stanley Lutts[2].
(1) Laboratorio de Fisiología y Biología Molecular de Plantas, INIA, La Cruz, CL
(2) Université Catholique de Louvain, BE
(3) Departamiento de Química, Universidad Técnica Federico Santa María, Valparaíso, CL
(4) Universidad Técnica Federico Santa María, CL
(5) Instituto de Producción y Sanidad Vegetal, Facultad de Ciencias Agrarias , Universidad Austral de Chile, Valdivia, CL

Pseudomonas syringae pv. tomato (Pst)is one of the most harmful bacteria in the industrial and agricultural production of tomato. This phytopathogen causes bacterial speck disease and, therefore, significant economic losses. The control the pathogen, antibiotics and highly toxic chemical compounds are generally used. The objective of this study evaluates the effects of the use of the tomato rootstock on the physiology and antioxidant defense mechanisms in the Old Limachino Tomato (OLT) grafted plant challenged to *Pst*. Rootstocks have been widely used to produce tomatoes, contributing to clean handling, especially in sensitive crop to environmental conditions such as the Limachino tomato. This genotype is highly susceptible to environments of biotic and abiotic stress.The experiment involved self-grafted (L/L) and grafted plants on INIA rootstock (L/R) under a completely random design. Plants were evaluated at 3 and 14 days after inoculations with *Pst*. Growth parameters and damage produced were assessed in plants. Furthermore the plant physiology, gene expression, antioxidant metabolism in the defense mechanism were studied. The plant defense response through the antioxidant mechanism and H2S synthesis in the aerial part was determined. Rootstock increased gene expression of antioxidant metabolism (GR, APX, CAT, SOD) and salicylic acid content in Limachino plant. On the other hand, rootstockreduced the MDA increase and bacterial growth in Limachino tomato. At 3 days after infection, significant differences between grafted plant types were observed, where L/L plants showed higher incidence, severity and bacterial CFU than L/R plants.

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Identification of placentary proteins of Cervico-Vaginal Brushing through Button Up Proteomics over the first trimester of pregnancy: a proof of concept

Francisco Alvarez[1], Mauricio Hernandez[1], Guillermo Nourdin [1], Pablo Saldivia[1], Cristian Vargas[2], Wendy Garín [2], Stecher Juan F [2], Elard Koch[1].
(1) Division of Proteogenomics, MELISA Institute , San Pedro de la Paz, CL
(2) Clinical Protocols, Division of Epidemiology , MELISA Institute , San Pedro de la Paz, CL

Recent publications have demonstrated the possibility of trophoblastic cells retrieval from cervical brushing of pregnancy patients since the fifth week. This strategy has the potential of early non-invasive study of placental dysfunction markers. At the present, research efforts to obtain trophoblast cells have focused on a genomic/ transcriptomics approach. We explore the possibility of identifying placental proteins in cervico-vaginal brushing during the first trimester of pregnancy. A sample of one voluntary pregnant women (9 gestational weeks) and non-pregnant control participated in this study. Cervico-vaginal brushings were collected using Rovers Cervex-Brush (DB) and transported in RPMI culture medium. Samples were centrifuged and cells lysed under standard procedures. After digestion with trypsin, peptides were quantified by IR and processed triplicated in a nanoHPLC (nanoElute) Mass Spectrometry using our TimsTOFpro instrument (Bruker). Protein identification was performed by Swissprot/Uniprot database (560,292 entries) for human taxonomy using the PEAKS studio X bioinformatic platform. We obtained 86,346 identified spectra and 11,055 peptides with a total of 1,206 proteins. After filtering by constitutive proteins from the cervico-vaginal tract and comparing with a reference proteome for placenta (ProteinAtlas). 15 placentary protein were identified. Of note, different isoforms of choriogonadotropin subunit beta were identified by mass spectrometry, indicating the suitability of this proteomics approach to find specific biomarkers of placenta. This proof of concept underlines the potential of using cervico-vaginal brushing to identify placental biomarkers by bottom up mass spectrometry. The technique is safe and minimally invasive. Further studies in different clinical conditions are warranted.
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Differential Expression of Lysyl Oxidases Enzymes in Diffuse and Intestinal Gastric Cancer Cell Lines

Isabel Vidal-Beltrán [2], Armando Rojas[1], Ramón Pérez-Castro[1], Carolina Añazco[1].
(1) Laboratorio de Investigaciones Biomédicas. Ciencias Preclínicas, Medicina, Universidad Católica del Maule, Talca, CL
(2) Ingeniería en Biotecnología, Ciencias Agrarias y Forestales, Universidad Católica del Maule, Talca, CL

Introduction:
Gastric cancer (GC) is one of the most common tumor pathologies in the world. Due to this fact, it is essential to identify new tumor biomarkers that allow differentiating between GC subtypes, facilitating the diagnosis and clinical treatment. Several reports has been related to lysyl oxidases (LOXs) enzymes with GC progression and then, we expect that one or more LOXs proteins act as potential biomarkers.

Methodology:
To achieve this goal, we used three cellular models: GES-1 (control), MKN45 (diffuse) and MKN75 (intestinal). The methodology involved the evaluation of LOXs expression (LOX, LOXL1, LOXL2, LOXL3 and LOXL4) by immunodetection.
Results: The studies indicate that unprocessed forms of LOX, LOXL1, LOXL2 are expressed in all cell lines. LOXL3 is not expressed in any cell lines, whereas the LOXL4 isoform is expressed just in the MKN74 cell line and it is not detected in MKN45 or GES-1.

Conclusions:
This finding indicates that the LOXL4 isoform would have a potential as a biomarker for the intestinal GC type, which differentiates it from diffuse GC. Further studies are needed to evaluate the potential of LOXL4 as a tumor marker in human clinical models.

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Mechanical unfolding of a knotted protein with molecular plug

Boris Arancibia-Antichivichi[1], Maira Rivera[1], Rodrigo Rivera[1], Andrés Bustamante[1], Mauricio Báez[1].
(1) Bioquímica, Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, CL

Knots are intricate topologies of proteins observed in about 2% of the structures deposited in the protein data bank (PDB). These proteins form a knot spontaneously and many molecular dynamics have been carried out to determine how these complex topologies are created. To contrast experiments with the folding mechanism predicted by molecular dynamics, we have used optical tweezers. However, although in theory optical tweezers can be used to untie a protein knot by pulling its structure from different points, this methodology cannot show directly if a molecular knot can be untied upon mechanical perturbation. To address this problem, we fused GFP to the C-terminus of a knotted proteins namely MJ0366. The idea is prevent that the knot of MJ0366 become untied upon mechanical unfolding. When MJ0366 was mechanically manipulated to untie the knot, the presence GFP decrease the molecular extension expected for the full unfolding of MJ0366. Moreover, the patter of refolding of MJ0366-GFP was similar to the observed for a mutant designed to tight the knot in MJ0366. Conversely in absence of the GFP, the molecular extension of MJ0366 fit with the expected value for the unfolding of MJ0366. These results suggest that GFP work as a molecular plug that preserve a knot in the denatured state of a protein and validate the methodology of optical tweezer to mechanically untie a protein knot.

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Molecular basis of a novel Glucokinase mutation which causes Maturity-Onset Diabetes of the Young type 2 discovered in a Chilean patient

Óscar Marcelo Aránguiz[1], Rodrigo Rivera[1], Pilar Durruty[2], Daniela Seelenfreund [1], Mauricio Báez[1].
(1) Bioquímica y Biología Molecular, Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, CL
(2) Endocrinología y Diabetes, Hospital Clínico, Universidad de Chile, Santiago, CL

Glucokinase (GK) is a key regulatory enzyme in the release of insulin from the pancreatic β-cell. Its high S0.5 and positive cooperativity for glucose modulate the increase in activity of the enzyme due to rises in glucose levels, which precede the release of insulin. Inactivating mutations in GK cause Maturity-Onset Diabetes of the Young type 2 (MODY2), a subtype of monogenic diabetes. Recently, a novel GK mutation (Gly448Asp) was discovered in a Chilean MODY2 patient. This mutation has not been characterized and its effects on the kinetics of GK could explain the associated pathogenicity. For the characterization, wild type (WT) human pancreatic β-cell GK and the Gly448Asp mutant were expressed using a heterologous system and purified by a single step of immobilized metal ion chromatography. GK activity was measured using a NAD+-coupled assay, and kinetic parameters were calculated using non-linear regression analysis. The mutant presents a kcat of 29.7±0.8[s-1], which is half of the wild type enzyme, and an S0.5 of 1.7±0.2 [mM] for glucose, a three-fold lower value. There were no changes in cooperativity for glucose and Km for ATP. The mutant lower kcat shows that, at physiological levels of glucose (approximately 5.5 [mM]), it displays a lower activity compared with the WT enzyme. In addition, the lower S0.5 for glucose leads to saturation conditions at physiological glucose levels. These altered kinetics could result in an enzyme that lacks the ability to increase its activity in response to changes in blood glucose, leading to alterations in insulin release.

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Substrate specificity and phylogenetic analysis of HMPP Kinases of the B1 vitamin biosynthesis pathways in bacteria

Gissela Araya[1], Pablo Cea[1], Victoria Guixé[1], Víctor Castro-Fernández[1].
(1) Departamento de Biología, Facultad de Ciencias, Universidad De Chile, Santiago, CL

Hydroxymethyl-pyrimidine-phosphate kinases (HMPPKs), encoded by the thiD gene, participate in the salvage and biosynthesis pathways of thiamine, phosphorylating 4-amino-5-hydroxymethyl-2-methylpyrimidine (HMP) to produce HMP-Phosphate (HMP-P) or HMP-Pyrophosphate(HMP-PP). HMP can also be phosphorylated by pyridoxal kinases (PLK), which participates in the synthesis of vitamin B6 and encoded by pdxK gene. HMPPKs and PLKs are homologous and belong to the vitamin kinases family. HMPPK from Escherichia coli is reported as a highly specific enzyme for HMP or HMPP. However, in this family, there are bifunctional enzymes denominated PLK/HMPP Kinases like, which are more structurally related to specifics HMPKs. These enzymes having a redundant activity compared to the canonical PLKs, phosphorylate pyridoxal and HMP. In this work, we performed a phylogenetic analysis with all available sequences annotated as thiD or pdxK. The results strongly suggest that the divergence between these enzymes occurred early during the evolution of this family, showing that the PLK/HMPPK-like appear from the HMPP kinases group. Besides this, we determine if the substrate specificity reported for orthologous proteins is a conserved trait in the HMPPKs. We assay the activity of these enzymes from Salmonella typhimurium and Thermus thermophilus, mesophilic and thermophilic organisms, respectively. These organism are phylogenetically distant with different evolutive pressure. The enzymes are not promiscuous with pyridoxal, pyridoxine and pyridoxamine, showing a high substrate specificity for HMP, which seems to be a conserved feature among them, suggesting that they participate only in the thiamine biosynthesis pathway.

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Effects of zinc on pulmonary hypertension in different models of hypoxia

Karem Arriaza[1], Julio Brito[1], Patricia Siques[1], Karen Flores[1], Daniel Aguayo[1], Silvia Arribas[2], Maria Del Carmen González [2], Angel Luis López De Pablo [2], Maria Rosario López [3].
(1) Institute of Health Studies, Universidad Arturo Prat, Iquique, CL
(2) Department of Physiology, Faculty of Medicine, Universidad Autónoma of Madrid, Madrid, ES
(3) Department of Preventive Medicine and Public Health, Universidad Autónoma of Madrid, Madrid, CL

Background:
High altitude has been associated with erythrocyte alterations, pulmonary hypertension, right ventricular hypertrophy (RVH) and oxidative stress. The effects of the combination of this conditions and exposure to metals such as zinc, on hematological, cardiovascular system and oxidative stress are not well known. The aim is evaluate zinc supplementation over different models of hypobaric hypoxia and association with MT, MTF1, PKCε and oxidative stress pathways.

Methods:
Wistar rats were exposed to simulated hypoxia in a hypobaric chamber at 428 Torr (4800m) for 30 days, randomly allocated in 3 groups: Chronic(CH), n=16; Intermittent(CIH), hypoxia 2 days/2 days normoxia; n=16; Normoxia(NX; n=16). Each group was divided into two (n=8) receiving intraperitoneally, either a Zn sulphate solution at 1% or just saline, every 4 days. Plasmatic and pulmonary zinc were measured by Atomic absorption spectrophotometry. HIF2α, MT, MTF1, and PKCε protein´s expression in lung tissue by Western Blot and lipid peroxidation by T-BARS.

Results:
Hypoxic groups: decreased weight, plasma zinc and pulmonary zinc. Higher RVH in CH(zinc), both CH(Zinc) and CIH(Zinc), increased Hct and Hb was seen without differences between them. Also hypoxic lung showed a protein overexpression of MT, MTF1, being higher in CH(zinc) group and PKCε in the CIH(zinc) group, along with increased lipidic peroxidation being higher in Zn groups.

Conclusion:
Simultaneous exposure to CIH and CH with zinc increases hematological and cardiovascular variables (RVH), which could be due to overexpression of MT and MTF-1 protein, and by effects of the oxidative stress.

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Loss of function of polycystin-1 induces metabolic dysfunction and mitochondrial fission in cardiomyocytes

Andrea Ramirez-Sagredo[1], Yáreni Ávalos Guajardo[1], Camila López-Crisosto[1], Jafet Ortíz-Quintero [1], Zully Pedrozo[2], Mario Chiong[1], Sergio Lavandero[1].
(1) Biochemistry and Molecular Biology, Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, CL
(2) Physiology and Biophysics, Faculty of Medicine, University of Chile, Santiago, CL

Introduction:
Polycystin-1 (PC1) is a membrane-bound protein described as a mechanosensor in cardiomyocytes. PC1 is an important regulator of cardiac function and mediates in mechanical stretch. Cells with loss-of-function of PC1 show dysregulation of Ca2+ signaling associated with impaired glycolysis and fatty acid oxidation. These data suggest a relationship between PC1 and mitochondrial function and metabolism. However, little is known whether these phenomena occur in cardiomyocytes.

Objective:
To determine whether PC1 regulates mitochondrial dynamics and proteins related with metabolic process.

Methods:
In vitro experiments were carried out in neonatal rat cardiomyocytes treated with PC1 siRNA. Mitochondrial morphology was determined using mitrotracker green and confocal microscopy.

Results:
In vitro experiments showed that PC1 knockdown increased the mitochondrial number per cell and decreased the average mitochondrial volume, suggesting the occurrence of mitochondrial fission. Also, we observed that this alteration is companies with changes in the expression of proteins that participate in the regulation of mitochondrial dynamics.

Conclusions:
The decrease of PC1 protein levels induced alterations in mitochondrial mass and mitochondrial fission. Considering the importance of this organelle in the cardiomyocyte, these alterations would be translating into an important cellular dysfunction.

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Role of the mitochondrial ubiquitin E3 ligase 1 (MUL1) in palmitate induced effects on insulin desensibilization and mitochondrial metabolism in L6 myoblasts

Karina Valeska Balboa[1], Macarena García[1], Rosemarie Mellado[2], Valentina Parra[1], Sergio Lavandero[1,3].
(1) Advanced Center for Chronic Diseases, Ciencias Químicas y Farmacéuticas, Universidad de Chile, santiago, CL
(2) Pontificia Universidad Católica de Chile, CL
(3) department of internal medicine (cardiogy division), Univesity of Texas Southwestern Medical Center, texas, CL

Introduction:
Circulating levels of free fatty acids (FFA) are increased in obese patients, leading to its accumulation in skeletal muscle and the development of insulin resistance. However, the full understanding of the mechanism behind this is still under study. MUL1 is a multifunctional ligase protein, with a ubiquitin ligase E3 activity, which, participates in ubiquitin transfer cascade reactions. It has been reported that AKT and MFN2 are regulated negatively by MUL1, Specifically, MUL1 ubiquitinates AKT and MFN2 causing their proteosomal degradation. MUL1 is increased after lipotoxic stress, but if MUL1 participates in metabolic regulation and in the insulin receptor signaling pathway is currently unknown.

Methodology:
We exposed L6 myoblasts to different palmitate concentrations to evaluate MUL1 levels.L6 myoblasts were exposed to palmitate (12.5nM) for 6 hours and in the last 15 minutes of the experiment a pulse of insulin (100nM) was added. The levels of AKT, p-AKT and MUL1 were analyzed by Western blot, as well as membrane potential mitochondrial by means of flow cytometry. Also, We used siRNA to dissect the role of MUL1.

Results:
In L6 myoblast, palmitate increased MUL1 protein levels. Besides, palmitate treatment decreased the levels of p-AKT and p-IR after the insulin pulse, which is consistent with insulin desensibilization. Moreover, palmitate caused a decrease in mitochondrial potential, that was prevented by a MUL1 siRNA.

Conclusion:
This data suggests that MUL1 is an important mitochondrial protein involved in the desensibilization to insulin triggered by palmitate, and in the maintenance of mitochondrial metabolism.

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Increased aminopeptidase A activity and renin angiotensin system disbalance in diabetes produces hypertrophy in cardiomyocytes

Isabel Bernales[1], Pablo Mendoza[1], Danilo Concha[1], Carlos Oyarzún[1], Rody San Martin[1].
(1) Instituto de Bioquímica y Microbiología, Facultad de Ciencias , Universidad Austral de Chile

Introduction.
Cardiovascular diseases (CVDs) are the main cause of death worldwide. Among its risk factors, diabetes remains important due to an increasing worldwide prevalence.Diabetic cardiomyopathy is characterized by diastolic dysfunction, hypertrophy and fibrosis. Studying diabetes pathogenesis in sterptozotocin-induced diabetic rats, we have evidenced the cardiac induction of Aminopeptidase A (APA), an enzyme that may modify the peptide arrange of the renin-angiotensin system (RAS), either by increasing the generation of bioactive peptides or by increased catabolism of peptides with cardioprotective action, such as Ang 1-7. We demonstrated the hypertrophic effect of peptides angiotensin 2-7 and 2-8, main products of APA,In H9C2 cardiomyocytes.

Methods.
Male rats had diabetes induced by administration of streptozotocin. APA activitywas determined in cardiac tissue homogenates, incubated with the fluorogenic APA substrate L-glutamic-β-naphtylamide. Fluorescence at 415nm was registered. The APA mediated activity is considered as the activity inhibited by Amastatin 10µM. The hypertrophy of H9C2 cardiomyocytes was measured by cell area following cytoskeleton staining using phalloidin (FITC).

Results.
APA activity in cardiac tissue of diabetic rats was more than two-fold than in control rats. Also, an increased APA stain was detected by IHQ in diabetes The peptides 2-7 and 2-8, which are the main products of APA activity, have a hypertrophic effect in the H9C2 cell line of cardiomyocytes.

Conclusions.
Increased cardiac Aminopeptidase A in diabetes is related to a RAS inbalance. Peptides angiotensin 2-7 and 2-8, main products of APA, produce hypertropia on the H9C2 cell line of cardiomyocytes

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Endogenous parvoviral element, Odegus4, is expressed as a protein in degu

Angelica Bravo[1], Luis Mercado[2], Gonzalo Mardones[3], Gloria Arriagada[1].
(1) Instituto Ciencias Biomédicas, Medicina, Universidad Andrés Bello, Santiago, CL
(2) Instituto de Biología, Ciencias, Pontificia Universidad Católica de Valparaíso, Valparaíso, CL
(3) Centro de biología celular y biomedicina, Universidad San Sebastián, Santiago, CL

Introduction.
Endogenous viral elements (EVEs) are viral-derived DNA sequences present in the genome of current species. Some of them possess open reading frames (ORF) that can express proteins with important roles in their host. Previously our laboratory demonstrated that an EVE derived from *Parvoviridae* family is transcribed in the liver of *Octodon degus*. This EVE, named Odegus4, contains an intact ORF that possess NS protein domains of adeno-associated virus, where NS is an essential protein for viral DNA replication. Furthermore, we demonstrated that in cells transfected with a plasmid encoding Odegus4, a protein with nuclear localization is expressed. Here we aim to determine the expression of Odegus4 as a protein in degu.

Methodology.
Antibodies against recombinant Odegus4 were generated and their capacity to recognize Odegus4 protein was tested by western blot of extracts from cells transfected with plasmids encoding Flag-Odegus4, GFP-Odegus4 and Odegus4-MycHIS. Then, these antibodies were used in western blot assays of protein extracted from various degu tissues.

Results.
We obtained two serums able to recognize Odegus4 in western blot assays, independently of the presence of a tag at amino or carboxy terminus. Using both antibodies we detected Odegus4 only in liver of degu.

Conclusions.
Our results show that Odegus4 is being translated to a protein in degu liver, opening a new line of research to investigate its possible biological functions, were giving resistance against parvovirus infections is a one strong possibility.

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Effect of RCAN1 overexpression on proliferation and DNA damage in induced pluripotent stem cells (iPSC) of subjects with Down syndrome

Francisco Javier Bravo[1,2], Sebastián Leiva-Navarrete[1,2], Carla Arias-Dúran[1,2], Valentina Parra[1,2].
(1) Advanced Center for Chronic Diseases (ACCDiS), Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, CL
(2) Autophagy Research Center, Odontología , Universidad de Chile, Santiago, CL

Introduction:
Down Syndrome (DS) is the most common autosomal aneuploidy, which is the product of an extra copy of chromosome 21 and is related to different neuronal and cardiac pathologies. DS patients present increased oxidative stress and, therefore, increased DNA damage; in addition to altered cell differentiation of neurons and cardiomyocytes. In humans, RCAN1, located on chromosome 21, is responsible of the enlarged and over functional mitochondria observed in DS iPSC. For this reason, the relationship of RCAN1 increased dosage with the proliferation and accumulation of DNA damage in these cells will be evaluated.

Methodology:
To analyze whether overexpression of RCAN1 modulates proliferation and DNA damage of disomic (2S) and trisomic (3S) iPSC, we will measure the expression of the proliferation marker Ki67 and the DNA damage product 8-Oxoguanin by means of immunofluorescence using an RCAN1 siRNA and an RCAN1 adenovirus.

Results:
The overexpression of RCAN1 in 3S iPSC induced an enhanced proliferation, which is reversed by the use of a siRCAN1. The RCAN1 adenovirus in the 2S iPSC increased their proliferation to levels similar to those observed in the 3S iPSC. 8-oxoguanin immunofluorescence showed cumulative DNA damage in iPSC 3S compared to 2S, which is dependent on the expression levels of RCAN1.

Conclusion:
RCAN1 overexpression regulates the increased proliferation and DNA damage observed in 3S iPSC.

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Mechanical untying of an artificial deeply knotted protein

Andrés Bustamante[1], Rodrigo Rivera[1], Maira Rivera[1], Mauricio Baez[1].
(1) Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, CL

Knotted topologies can remain in the unfolded states of knotted proteins even in strong denaturant conditions. Nonetheless, the dynamics of knots in protein 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) in order to untie its polypeptide chain using optical tweezers. Force-ramp experiments showed single unfolding and refolding transitions. However, unfolding events were grouped in two clusters indicating two different unfolded sates. One ($U_{expanded}$) is observed at low forces and showed a contour length (ΔLc) in agreement with the expected value for the fully unfolded-unknotted protein (~30 nm). The other unfolded state ($U_{compact}$) is observed at higher forces and showed shorter ΔLc (~21 nm). These data suggest that the protein can be fully unfolded-untied ($U_{expanded}$), or the protein remains trapped in a knotted intermediate ($U_{compact}$). Nevertheless, the refolding events showed only one cluster with a unimodal folding force distribution. This suggest that the two unfolded states have the same refolding energy barrier. Hence, it's not clear if the protein can be unknotted during the mechanical perturbation. Our data suggest that the untying of the polypeptide chain is not coupled to the unfolding. We propose that the unknotting process have a high energy barrier due to the deepness of the knot present in 2ouf-knot.

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Sobitol metabolism in non-*Rosaceae*: studies of a putative aldose-6-phospate reductase in *A. thaliana*

Pamela Cabedo[1], Michael Handford[1].
(1) Centro de Biologia Molecular Vegetal, Ciencias, Universidad de Chile, Santiago, CL

In plants, polyols such as sorbitol have been linked to protective roles due to their reduced state and consequently their ability to act as a source of reductive power against reactive oxygen species. Sorbitol is also capable of acting as a compatible solute, and this polyol has been linked with higher tolerance to different forms of abiotic stress including cold and saline stress, as well as binding and mobilising the micronutrient, boron. In *Rosaceae*, sorbitol is the primary photosynthate and is synthesised in source organs from glucose-6-phosphate by aldose-6-phosphate reductase (A6PR), being later translocated via phloem to sink organs where it is oxidised to fructose by sorbitol dehydrogenase (SDH). *Arabidopsis thaliana*, while not a member of the *Rosaceae*, possesses two open reading frames encoding putative A6PRs, *At2g21250* and *At2g21260,* named *AtA6PR1* and *AtA6PR2*, respectively. *AtA6PR2* possesses lower expression levels when compared to *AtA6PR1* and its role in *A. thaliana* is still unknown. In order to establish this function, stably transformed *A. thaliana* lines expressing AtA6PR2 under a constitutive promotor, have been generated. Expression of the AtA6PR2 fusion product has been determined at transcript and protein levels. Moreover, stable transformation of *atsdh1-1* lines with *AtA6PR2* resulted in marked phenotypical differences compared to other *AtA6PR2*-expressing lines.

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The bZIP transcription factors that belong to group C and S participate as negative regulators of endocytic traffic in *Arabidopsis thaliana* roots

Francisca Cabello [1], Lorena Pizarro[1], Johannes Hanson[2], Lorena Norambuena[1].
(1) Department of Biology, Faculty of Sciences, Universidad de Chile, Santiago, CL
(2) Umeå Plant Science Centre, Department of Plant Physiology , Umeå Universitet, Umeå, SE

Under adverse conditions, plants display a variety of mechanisms at molecular and cellular level that translate into physiological processes allowing them to respond appropriately. One of these responses is the generation of lateral roots. In our laboratory, we had described an important role of endocytic trafficking in the mechanism of organogenesis of lateral roots. At molecular level, we have seen that the transcription factor bZIP25 is a negative regulator of endocytosis. This result was obtained by using a loss-of-function mutant of this factor, the *bzip25-2*. This transcription factor is a member of a big gene family classified on groups based in their molecular structure. The aim of this work was to determine whether other member of bZIP groups functionally related to bZIP25, share the role in endocytic trafficking. For this purpose, loss-of-function mutants in genes that encode the transcription factors of group C (bZIP25, bZIP10 and bZIP63) and S (bZIP53) of bZIP family were studied. To assess the endocytic trafficking to the vacuole in mutant plants we used the fluorophores FM1-43 and FM4-64. The results indicate that the lack of function of *bZIP10*, *bZIP63* and *bZIP53* caused an increase in the endocytic rate, similar phenotype displayed by *bzip25-2* mutant. However, the loss of function of bZIP28 (group B) did not display an endocytic phenotype. These results suggest the participation of the bZIP25, bZIP10, bZIP63 and bZIP53 in endocytic trafficking in Arabidopsis roots.

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Molecular modeling analysis of Single-nucleotide polymorphisms (SNPs) of *Caligus rogercresseyi*

Calderón Juan Pablo [1,2], Yorley Duarte[1], Cristian Gallardo-Escárate[3], González-Nilo Fernando D.[1,2].
(1) Center for Bioinformatic and Integrative Biology, Ciencias de la Vida, Universidad Andrés Bello, Santiago, CL
(2) Instituto Milenio Centro Interdiciplinario de Neurociencia de Valparaíso, Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, CL
(3) Laboratorio de Biotecnología y Genómica Acuícola, Departamento de Oceanografía, Universidad de Concepción, Concepción, CL

Caligus rogercresseyi is one of the most significant agents affecting farmed salmonids around the world, with Chile as one of the most affected countries. This parasite causes several damages, including salmon's skin injuries and loss of the physical and microbial protective function which creates immunological undermining. Control strategies on C. *Rogercresseyi* rely mainly on the use of pesticides, but unfortunately, the resistance has seen an increase.

Due to this problem, we considered the importance to study and extend the knowledge to a structural level of 30 new SNPs obtained experimentally from C. *Rogercresseyi*. These targets are those that have a special relationship with drugs (azamethiphos, deltamethrin, cypermethrin). At present, no protein structure has been reported, experimentally obtained from *Caligus*. This lack of information forces to make in silico models to create a structural characterization of the targets found in the laboratory. To achieve this aim, we used several computational tools like homology modeling (simple, multiple alignments and consensus modeling), molecular dynamics simulation and molecular docking to understand to structural level the interactions and residues that lead the feasible resistance to antiparasitic drugs. From the results, we have analyzed the kind of mutations arising from SNPs, studying their disruptive or non-disruptive characteristics that may be altering the union of the protein with the antiparasitic drug.

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17-β estradiol regulates hypertrophy and MUL1 in cultured rat cardiomyocytes

Calle-Chalco Ximena E [1], Sebastian Leiva-Navarrete[1], Valeria Garrido-Moreno[1], Troncoso Mayarling F[1], Valentina Parra[1], Sergio Lavandero[1,3,2].
(1) Advanced Center for Chronic Diseases (ACCDiS), Faculty Chemical & Pharmaceutical Sciences & Faculty of Medicine, University of Chile, Santiago, CL
(2) Corporación Centro Estudios Científicos de las Enfermedades Crónicas (CECEC), CL
(3) Cardiology Division, Faculty Chemical & Pharmaceutical Sciences & Faculty of Medicine, University of Texas Southwestern Medical Center, Dallas, Texas, , US

Introduction:
Cardiovascular disease risk is higher in men than in premenopausal women of the same age, but this female advantage is lost after menopause. This observation has led to ask whether decreased estrogen synthesis could be associated with the development of cardiac hypertrophy, a process characterized by increases in cardiac size, protein synthesis and in the expression of fetal gene program together with a decreased cardiac energy metabolism The multifunctional ubiquitin E3 ligase protein of the outer membrane mitochondrial MUL1 alters the fine balance between mitochondrial fission and fusion and mitochondrial energy function by the ubiquitination of mitofusin 2 and Akt and by the sumoylation of Drp1. However whether estrogen controls cardiomyocyte hypertrophy by regulating MUL1 remains not understood.

Aims:
to **s**tudy *in vitro* the effect of 17-β estradiol on cardiomyocyte hypertrophy and to investigate if whether estradiol prevents the increase in MUL1 protein levels observed in hypertrophied cardiomyocytes.

Methods and results.
Cultured neonatal rat ventricular myocyte (NRVM) were preincubated with or without 17-β estradiol (E2, 100 nM) prior to the treatment with 10 uM norepinephrine (NE) for 24 h. NE increases protein levels of atrial natriuretic peptide (ANP) and MUL1 assessed by western blot as well as cardiomyocyte area (using rhodamine-phalloidin staining). All these parameters were decreased with the previous treatment with E2.

Conclusions:
In vitro, 17-β estradiol decreases cardiomyocyte hypertrophy markers and MUL1. However it remains to be study the link between both findings.

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Role of the carrot phytochrome interacting factor 3 (*DcIF3*) in carotenoid synthesis in *Daucus carota*

Jonathan Canales[1], Felipe Durán[1], Nicolás Arancibia[1], Christian González[1], Claudia Stange[1].
(1) Biología , Ciencias, Universidad de Chile, Santiago, CL

Carotenoids are pigments involved in photosynthesis, photoprotection and plant hormone synthesis. Carotenoid synthesis is induced by light through the signal transduction mechanism induced by photoreceptors in photosynthetic tissues. *Daucus carota* (carrot) accumulates high levels of carotenoids in its storage root that develops in darkness, contrary to other plants. An RNA-seq analysis was carried out comparing roots grown in dark (R/D) and in the presence of light (R/L). Some light-regulated genes, such as PHYTOCHROME INTERACTING FACTOR 3 (PIF3), were up-regulated in dark grown roots. In *Arabidopsis, PIF3* codes for a transcription factor that represses photomorphogenesis and carotenoid synthesis in darkness. PAR1 induces carotenoid synthesis by sequestering PIF3. In carrot, *DcPIF3* and *DcPAR1* genes expresses mostly in R/D than in R/L during its development. *In silico* analysis showed that *DcPIF3* presents 48% of identity to *AtPIF3* containing conserved DNA and protein binding domains. The direct binding of DcPIF3and DcPAR1 was assessed by a Bimolecular Fluorescent Complementation Assay (BIFC). Arabidopsistransgenic lines that overexpress *DcPIF3* when growing in the dark present higher growth of the aerial parts, a repression in the expression levels of *AtPSY* and *AtPAR1* genes, a decrease in total carotenoids and a greater accumulation of DcPIF3 protein than when exposed to white light. These results are consistent with the role described for AtPIF3 as a repressor of photomorphogenesis. Taken together, our results suggest that *DcPIF3* codes for a functional protein which in carrot tap root could be inhibited by DcPAR1 producing an increase in carotenoid content.

Structural characterization of 17β-estradiol: a new TRPV1 modulator

Jonathan Canan[1,2], Karina Carvajal[3], Sepúlveda Romina V.[1], Ignacio Diaz-Franulic[2], Ramón Latorre[2], Pablo Olivero[3], Karen Castillo[2], González-Nilo Fernando D.[1,2].
(1) Center for bioinformatics and integrative biology, Ciencias de la Vida, Universidad Andrés Bello, Santiago, CL
(2) Instituto Milenio Centro Interdisciplinario de Neurociencia de Valparaíso, Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, CL
(3) Laboratorio de Estructura y Función Celular, Facultad de Medicina, Universidad de Valparaíso, Valparaíso, CL

The transient receptor potential vanilloid type 1 (TRPV1) channel is a polymodal receptor working as a molecular integrator of multiple physiochemical stimuli, including ligands like capsaicin. TRPV1 has an important role in THE pain pathologies, being a relevant pharmacological target for THE pain relief. 17 β-estradiol (E2) is a key hormone that participate in the regulation and differentiation development of several tissues, this include male and female reproductive tracts, mammary glands and, skeletal and cardiovascular system. E2 also promotes neuronal protection during brain ischemia, and oxidative damage, this is an obliterated effect when TRPV1 blockers are present. In order to explore if E2 is able to modulate TRPV1 activity, we measured the activity of this channel by patch-clamp technique in presence of E2, and we observed that E2 activates the channel. To know possible structural interactions between E2 and the TRPV1 channel, we explored the potential structural configurations of TRPV1 with 17β-estradiol, with capsaicin and also with both ligands, under different conditions, using a strategy based on docking simulations, and the protein-ligand binding affinity (MMGBSA) from a significant molecular dynamics simulation. We observed that E2 molecule binds to TRPV1 in the vanilloid pocket, i.e, the pocket binding site is shared by 17β-estradiol and capsaicin. We have identified possible interacting relevant residues, and by site-directed mutagenesis, we determined its relevance on TRPV1 activation by E2. All these results will contribute to the understanding TRPV1 activation by steroidal hormones and it could possible lead to the design of new pain modulators.

Instituto Milenio Centro Interdisciplinario de Neurociencia Valparaíso, ICM-MINECON P09-022-F. Fondecyt Regular 1170733. Center for Bioinformatics and Integrative Biology, Facultad Ciencias de la Vida, Universidad Andrés Bello.

Chronic exercise and myostatin expression in mouse aorta

Nicole Cancino-Arenas[1], Fernanda Sanhueza-Olivares[1], Ignacio Norambuena-Soto[1], Francisco Díaz-Castro[2], Paulina Villar-Fincheira[1], Tomás Hernandez-Díaz[1], Matías Monsalves-Alvarez[1,2], Rodrigo Troncoso[2], Mario Chiong[1].
(1) ACCDiS. Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, CL
(2) Laboratorio de Investigación en Nutrición y Actividad Física, Instituto de Nutrición y Tecnología de los Alimentos, Universidad de Chile, Santiago, CL

Chronic exercise induces changes in the cardiovascular system to meet the increased metabolic requirements. Skeletal muscle is the main target and effector of physical activity. Skeletal muscle secretes myokines to coordinate muscle activity with other organs to meet the high energy demand during exercise. Myostatin is a myokine described as a negative regulator of skeletal muscle growth. Its mRNA and plasma levels decrease upon chronic exercise. Whether myostatin is expressed and involved in vascular remodeling induced by exercise has been not described yet. The objective of this work is to determine if chronic exercise modifies the expression of myostatin in mouse aorta. C57BL6 mice were exercised 40 minutes/day at 70% of their maximum speed, 5 days per week for 30 days. Two weeks after the end of the exercise protocol, mice were euthanized and the aortas were obtained. As a control, sedentary mice were used. Aortic mRNA was extracted and myostatin mRNA was quantified by RT-qPCR. The results showed that the exercised mice showed an increase in their maximum aerobic speed, a lower final body weight with no changes in food intake. In aorta, myostatin mRNA expression was detected. However, chronic exercise did not modify myostatin mRNA levels as compared to sedentary mice. It was concluded that chronic exercise does not modify the expression of myostatin in mouse aorta.

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Tridimensional multispecies biofilm model for chronic wounds

Camila Cárdenas[1], Tamara González[2], Patricia García[2], Mario Vera[1,3], Tomás Egaña[1].
(1) Instituto de Ingeniería Biológica y Médica, Ingeniería, Medicina y Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, CL
(2) Microbiología y Parasitología, Medicina, Pontificia Universidad Católica de Chile, Santiago, CL
(3) Ingeniería Hidráulica y Ambiental, Ingeniería , Pontificia Universidad Católica de Chile, Santiago, CL

Introduction:
Chronic wounds cannot heal due to impaired tissue regeneration, caused by pathogenic biofilm-infections that provoke chronic inflammation, hypoxia and skin degradation. Biofilm-infections are persistent given their tolerance to broad-spectrum antibiotics and their multispecies composition, as shown by clinical isolates from chronic wounds. Representative *in vitro* multispecies biofilm models are crucial for screening new anti-biofilm strategies with therapeutic potential.

Objective:
Establish a tridimensional multispecies biofilm model over artificial skin scaffolds, with broad-spectrum antibiotic tolerance.

Methodology:
Bacteria (*Pseudomonas aeruginosa, Staphylococcus aureus*, *Enterococcus faecalis*) were inoculated over artificial skin scaffolds, and incubated in M9 media during 24 h at 30°C. Samples were analyzed by fluorescent lectins binding analysis (FLBA) and CLSM, CFU counting, and MALDI-TOF mass spectrometry. For antibiotic tolerance, biofilm-containing scaffolds were incubated with ciprofloxacin 100 µg/ml or gentamicin 200 µg/ml during 24 h at 30°C, and XTT-reduction assay was performed. For statistical analysis, data were analyzed by two-way ANOVA and post-hoc test.

Results:
Cultures containing the three bacterial species formed a multispecies biofilm over the scaffold fibers after 24 h. Attached bacteria were not released from the biofilm, neither by PBS washes nor gauze drying steps. All three species were present, and total bacterial load ranged within 10^5-10^6 CFU/g. Compared to planktonic bacteria, biofilm-containing scaffolds tolerated both ciprofloxacin and gentamicin.

Conclusions:
Under specific conditions, three bacterial species interact with the scaffold fibers forming a tridimensional biofilm, with high bacterial load and broad-spectrum antibiotics tolerance. This biofilm model can be used for *in vitro* screening of novel treatments for chronic wounds.

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Comparative analysis of the linker proteins Hmo1 and Hho1

Roberto Amigo, Carlos Farkas, **Natalia Cartes**[1], Gutiérrez José L[1].
(1) Departamento de Bioquímica y Biología Molecular , Facultad de Ciencias Biológicas , Universidad de Concepción, Concepción, CL

Higher-order chromatin structure is stabilized by association of linker histone H1. In Saccharomyces cerevisiae, Hho1 protein has the greatest sequence similarity to H1. However, its role as linker histone is still controversial. Some studies have postulated that the protein Hmo1 could function as linker histone. However, Hmo1 is commonly considered a yeast high mobility group box (HMGB) protein, and HMG proteins are known as factors displaying functions which are opposed to those of linker histones. In this context, we have previously shown that Hmo1 stimulates nucleosome remodeling activity of ATP-dependent chromatin remodeling complexes in vitro, including the SWI/SNF complex. Additional studies suggest that Hmo1 plays a role in assisting the loading of this complex onto gene regulatory regions. Considering these discrepancies, we wanted to get further insight into the roles of Hmo1 and Hho1. Different in vitro and bioinformatic approaches were used. Analysis of ChIP-seq data for Hmo1, Hho1 and SWI/SNF subunits showed preferential binding of Hmo1 and SWI/SNF to long nucleosome-free regions, while Hho1 displays a more dispersed pattern. Comparison of occupancy levels to transcriptional activity, obtained from NET-seq data, shows that both Hmo1 and SWI/SNF preferentially occupy the promoter region of genes with the highest transcriptional activity, while Hho1 is more broadly distributed. GST pull-down assays show physical interaction of Hmo1 with SWI/SNF and other remodeling complexes. Our analyses support a role for Hmo1 which is closer to an HMG protein than to a linker histone.

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Substrate Specificity and Sequence Dependence of a Model ATPase-like Catalytic Amyloid

Claudio Castillo-Cáceres[1], Eva Durán-Meza[1], Esteban Nova[2], Rodrigo Diaz-Espinoza[2], Octavio Monasterio[1].
(1) Biología, Ciencias, Universidad de Chile, Santiago, CL
(2) Biología, Química y Biología, Universidad de Santiago de Chile, Santiago, CL

Amyloids are highly ordered protein aggregates with an elongated architecture that is stabilized by a β-sheet structural core. Though classically associated to pathology, reports on amyloid with functional roles have steadily emerged. Moreover, the recent discovery that amyloids formed with rationally designed small peptides can exhibit catalytic reactivity has opened up new opportunities in both biology and biotechnology. The activities require the binding of divalent metals, giving rise to active metal-amyloid complexes. Previous work from our group showed that self-assembled amyloids with a peptide containing the catalytic sequence from a nucleotydiltransferase (SDIDVFI) exhibited hydrolytic catalytic activity of this peptide in the amyloid state that is dependent of Mn2+, mimicking an ATPase-like enzyme. We performed a kinetic characterization of this activity using different substrates (ATP, CTP, UTP, GTP and dATP) in order to understand the specificity of the Mn2+-amyloid complex. Our results showed no significant differences on the kinetic parameters *KM*, *Vmax*,and *kcat* except for dATP that exhibited a fivefold increase on these constants. We also studied the contribution of the aspartate residues on the activity of peptide SDIDVFI by analyzing the ATPase-like activity of three mutants: SAIDVFI, SDIAVFI and SEIEVFI. The results showed a remarkable deviation on Km and Vmax values for peptide SDIAVFI, suggesting a significant role for the second aspartate on the observed ATPase-like activity. Overall, our results should contribute towards a systematic understanding of the emerging activity observed in these metal-amyloid complexes that will help the design of future catalytic amyloids with biotechnological and biological applications.

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A structural and conformational study of Adenylate Kinase using steered molecular dynamics

Castillo Carlos A. [1], Esteban Vöhringer-Martinez[1].
(1) Quantum Chemistry & Molecular Modeling group, Facultad de Ciencias Químicas, Universidad de Concepción, Concepción, CL

Adenylate kinase (AK) is an enzyme that has been postulated to suffer a partial unfolding/refolding event associated with the open/closed conformational change in the catalysis. The protein is formed by three subdomains: the core subdomain that is important for overall stability, the ATPlid and AMPlid subdomains that have the ATP and AMP substrate binding sites. We study with computer simulations which region of AK is the first to unfold under an external applied force to address this unfolding/refolding event. *In silico* pulling simulations were carried out for the AK wyld type of *A. aeolicus* (AKwt) and V117G/L162G double mutant. These systems were simulated using steered molecular dynamics (SMD) pulling the alpha-carbons of residues V145 and Y52 in opposite directions (force constant: 1000 pN/nm and a pulling velocity of $2.5*10^{-5}$ nm/ps. Our results of various representative conformations show that the ATPlid is the first domain to unfold for both AKwt and AK double mutant. At a pulling distance of about 5.5nm, AKwt presents a meta-stable state in which the ATPlid subdomain is partially unfolded. The AK double mutant, however, shows no meta-stable state and an increased loss of secondary structure in the ATPlid subdomain. This observations were confirmed further by experimental single molecule pulling experiments. On this ground, we can say that SMD simulations provide atomistic information about AK unfolding as consequence of external forces that allow a better interpretation of the experimental data.

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Molecular analysis of four variants of the *Stenocereus stellatus* species

Clara Cervantes-Arista[1], José Alberto Mendoza-Espinoza[2], Angélica Román-Guerrero[3], Fernando Díaz De León-Sanchéz[4], Edgar Sierra-Palacios[2].
(1) Biotechnology, Universidad Autónoma Metropolitana Unidad Iztapalapa, CDMX, MX
(2) College of Sciences and Humanities, Campus Casa Libertad, Universidad Autónoma de la Ciudad de México, CDMX, MX
(3) Group of Biochemistry of Macromolecules-Department of Biotechnology, Universidad Autónoma Metropolitana Unidad Iztapalapa, CDMX, MX
(4) Laboratory of Post-harvest of Plant Genetic Resources and Natural Products-Department of Health Sciences, Universidad Autónoma Metropolitana Unidad Iztapalapa, CDMX, MX

Introduction.
Some research on *Stenocereus stellatus* has identified some of its variants as important sources of compounds with potential uses in the industry. Since the fruiting time is 6 years, an early identification that helps in the selection of the variant of interest is convenient. Molecular marker analysis has been a successful tool for the identification of species in a wide variety of plants. Therefore, this research aims to use molecular analysis in the identification of the different variants of *Stenocereus stellatus*.

Methodology.
Variants were analyzed; white, red, orange and purple. DNA extraction, amplification of the markers (*psb*A-*trn*H and *mat*K) were performed by PCR. The amplified ones were cloned in the PJET plasmid, the transformation process was carried out in *Escherichia coli* DH5α. Finally, the cloned products were sequenced. These were analyzed in MEGA-X including two species of the genus *Stenocereus* and *Opuntia ficus* indica.

Results.
When comparing the sequences of the four variants of *Stenocereus* and that of *Opuntia ficus*, it was observed that with both markers it is possible to differentiate these two species at the genus level. It was observed that the sequences of the four variants show a difference in both markers, thus achieving their molecular identification.

Conclusions.
With this study it has been possible to identify the four variants through the *psb*A-*trn*H and *mat*K markers, it was also established that with both markers it is possible to differentiate at the gender level.

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Super-stable ECE-1c promotes a cancer stem cell phenotype in lung cancer cells

Cristopher Chavez[1], Trigo Cesar A[1], Pablo Perez[1], Manuel Varas[2], Francisco Aguayo[1], Julio Tapia[1].
(1) Basic & Clinic Oncology, Faculty of Medicine, Universidad de Chile
(2) Centro de biología molecular y biomedicina, Facultad de Medicina y Ciencias, Universidad San Sebastián, Santiago, CL

Lung cancer is one of the deadliest cancers in Chile and worldwide. Its current platinum-based chemotherapy is ineffective because of its high resistance, which may be related to enrichment of a cancer stem cells (CSCs) population that generate recurrence. A super-stable form of endothelin-converting enzyme-1c (ECE1cSS), a membrane metalloprotease,has been shown to enhance CSC-like characteristics in colorectal cancer cells, such as drug resistance, invasion, tumor growth, etc. However, whether this ECE1cSSprotein also promotes a CSC-like phenotype in lung cancer cells is unknown. A549 adenocarcinoma cell clones expressing FLAG-tagged ECE1c wild-type or super-stable (ECE1cSS) mutant were used. Protein stability of all ECE1c forms was performed by using the translation inhibitor CHX and/or CK2 inhibitor CX-4945. Cancer stem cells features were evaluated by detecting surface markers CD133 and CD44 by flow cytometry, expression of stemness genes by RT-qPCR, and sphere-formation assay. Drug resistance was evaluated by MTS in presence of cisplatin and side population assays. ECE1cSS displayed a higher stability than WT in A549 cells. ECE1cSS-expressing cells were highly double-positive for CD133+ and CD44+ superficial markers, as well as showed elevated levels of stemness mRNAs and improved sphere-formation capacity. Also, ECE1cSS-expressing cells had an enhanced resistance to cisplatin, which was consistent with elevated mRNA levels of some ABC pumps. Super-stability of ECE1c promotes a CSC-like phenotype in lung adenocarcinoma cells. These lung CSC-like cells have improved properties which are characteristics of aggressive CSC cells, such as enhanced resistance to cisplatine which is a traditional drug used for therapy of this cancer.

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Probing the elasticity of the Giant Titin

Fernanda Contreras[1], J Andrés Rivas-Pardo[1].
(1) Center for Genomics and Bioinformatics, Facultad de Ciencias, Universidad Mayor, Santiago, CL

Titin is the third filament in muscle. Whereas the thin filament—actin—and thick filament—myosin—establish the generating force system of the sarcomere, the titin filament determinates the passive elasticity of muscles. Titin is a giant filamentous protein, consisting in approximately 300 immunoglobulin-like (Ig) domains that anchors and communicates the Z-disk with the M-band of the sarcomere. Nevertheless, only ~100 Ig domains, belonging from the I-band region, are responsible of the elasticity of the muscle. Recent findings have proposed that a single mutation on the 10th Ig domain from the titin I-band—Tre16Ile—triggers myocardial diseases. Here, we have engineered a polyprotein based in the I10 titin domain, which we have repeated eight times and included a CysTag for protein anchoring. Through AFM-based force spectroscopy we observed that the folding ratio of I10 domain is low compare with other Ig titin domains; after a mechanical unfolding I10 domain is prone to acquire a random coil state which lacks of mechanical stability. Furthermore, preliminary data indicate that the folding can be modulated by the action of muscle chaperones. Together, our findings suggest a critical role for the 10th titin domain which could be related to myocardial dysfunction by impairment of titin elasticity; after a mechanical challenge during muscle stretching I10 fail to obtain the native state, but could be reach by the action of muscle chaperones.

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Biophysical characterization of the heterodimerization between the DNA–binding domains from human FoxP1 and FoxP2 transcription factors

Ricardo Coñuecar[1], Pablo Villalobos[1], César Ramírez-Sarmiento[2], Exequiel Medina[1], Jorge Babul[1].
(1) Departamento de Biologia, Facultad de Ciencias, Universidad de Chile, Santiago, CL
(2) Institute for Biological and Medical Engineering, Pontificia Universidad Católica de Chile, Santiago, CL

Forkhead box P (FoxP) are members of the human Fox family of transcription factors (FoxA-FoxS), involved in diverse processes such as organogenesis, metabolism and immune system regulation. The FoxP subfamily consists of four proteins (FoxP1−4), which, differently to the other subfamilies, dimerize via domain swapping using their DNA-binding domain. Specifically, FoxP1 and FoxP2 have been studied due their functional role in different human diseases. Both proteins share 88% sequence identity but have different dimerization properties, with *K*D values ranging from µM to mM for FoxP1 and FoxP2, respectively. Several reports indicate that FoxP1 and FoxP2 can form heterodimers *in vivo* for transcriptional regulation, being relevant in lung and esophageal development. However, the role of the DNA-binding domain and its DNA ligand in this association has not been elucidated. In this context, we evaluated the feasibility of FoxP1-FoxP2 heterodimerization *in vitro* characterizing their biophysical properties and the effect of DNA in the monomer-monomer association. Equilibrium measurements indicated that heterodimerization takes place under the conditions assayed and, surprisingly, the K_D ascertained is in the same order of magnitude as that for FoxP1 homodimerization, in notable contrast with FoxP2 homodimerization. Moreover, van't Hoff analysis revealed differences in the dissociation enthalpy, with a decrease in the dimeric contacts in the heterodimer, with respect to the FoxP1 homodimer. Finally, when a specific DNA for FoxP1 was used, only its homodimerization was affected, suggesting that interactions between each monomer and its ligand are needed to modulate the dimerization event

FONDECYT 1170701

Expression assessment of two isoforms of *Solanum lycopersicum* lipoyl synthase in tomato

María Paz Covarrubias[1], Michael Handford[1].
(1) Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, CL

Functional foods have basic nutritional importance, and also beneficial effects by adding different components like probiotics or vitamins. In general, oxidative stress can generate reactive oxygen species which in high levels can damage DNA, lipids and proteins. To control these processes, biological systems possess several antioxidant mechanisms such as antioxidant molecules. Among these, lipoic acid is unique because of its extremely powerful antioxidant capabilities. Lipoic acid is amphipathic, acts in both oxidised and reduced forms, and regenerates other antioxidants. However, lipoic acid is also a cofactor that is incorporated into several enzymes, including E2 subunits of pyruvate dehydrogenase (PDH) and α-ketoglutarate dehydrogenase (kGDH) complexes, which in turn belong to the TCA cycle. PDH is also found in plastids where it is required for fatty acid synthesis. The lipoylation process occurs via two routes in both plant organelles; *de novo* synthesis from fatty acids, and the salvage of free lipoate. Common to both pathways is the addition of two thiol groups into an octanoyl precursor already attached to target subunits. This reaction is catalysed by lipoyl synthase (LIP1). In order to obtain a tomato fruit with higher antioxidant capability, in the present work, mitochondrial (*SlLip1*) and plastidial (*SlLip1p*) *lipoyl synthase* genes from *Solanum lycopersicum* are being expressed under the fruit-specific promoter of the tomato *polygalacturonase* (*Slpg*) gene which is expressed during fruit ripening. The expression pattern of *SlLip1* and *SlLip1p* in wild-type and transiently transformed tomato fruits is being assessed.

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Cytokinin and auxin modulation is key to the plant developmental changes in response to A *nodosum* biostimulants in A. *thaliana*

Josefa Del Pozo[1], O´Brien José Antonio[1].
(1) Molecular Genetics & Microbiology, School of Biological Science, Fruit Production and Enology, School of Agricultural and Forest Sciences, Pontificia Universidad Católica de Chile, Santiago, CL

It has been shown that *Ascophyllum nodosum* derived biostimulants induced a hormonal-like activity on plants, specifically a cytokinin-like effect. However, little is known about the mechanism of action of these biostimulants and their role in the modulation of phytohormones throughout the plant. Two commercial biostimulants derived from A*. nodosum* extracts were used to evaluate the phenotypic response and the hormonal signaling induced by the biostimulant in the model organism *Arabidopsis thaliana*. For the phenotypic analysis, the root length, lateral root density, fresh and dry weight of roots and shoot, rosette area, and the rosette leaf number were measured. Reporter lines carrying GUS or GFP with the promoters for biosynthesis, response, and signaling related genes of cytokinin and auxin were used to evaluate the tissue-specific hormone signaling responses. Our preliminary results show that the biostimulant affects the signaling of auxin and cytokinin response genes in the roots, which could be modulating the changes observed in the development of the roots and the plant as a whole.

Transcriptome characterization of intestines of adult zebrafish males fed with soybean and fish meal protein-based diets

Nicole Diaz[1,2], Valenzuela Luis V.[1,2], Pacheco Sebastián A[1,2], Natalia Lam[1], Cristian Araneda[1], Pilar Ulloa[1,3].
(1) Laboratorio de Genética y Biotecnología en Acuicultura , Facultad de Ciencias Agronómicas , Universidad de Chile, Santiago, CL
(2) Escuela de Bioquímica , Facultad de Medicina y Ciencias, Universidad San Sebastián, Santiago, CL
(3) Facultad de Medicina Veterinaria y Agronomía, Universidad de Las Américas, Santiago, CL

The limited availability of fishmeal in aquaculture has forced the industry to find alternative protein sources, and soybean meal (SBM) is commonly-used substitute. However, in cultured fish and zebrafish, SBM induces intestinal inflammation, interfering with health and growth. Under this scenario, main challenges for aquaculture are to successfully feed and cultivate herbivore diet-tolerant fish strains. Nowadays, it is possible the integration of nutrition and genomics analysis through nutrigenomics approach, helping to understand the effect of diet on gene expression. Our approach was to feed two populations with the same genetic background; one set of 19 families was fed with a control fishmeal diet (100FM) and the other set of 19 families was fed with soybean meal diet (50SBM). Males' intestine tissue from the 5% in both tails of the weight normal distribution from a population fed with 50SBM were selected (low and high growth; 50 mg vs 180 mg, respectively) to compare with males of average growth after feed with 100FM diet to carry out RNA-seq assays to evaluate transcriptomic differences. Statistical analysis showed 107 and 45 differentially expressed genes (DEGs) in low and high growth individuals respectively, regarding to control diet. In both cases, the top-ten Biological Process in which DEGs were involved were the same, including "primary metabolic process", "nitrogen compound metabolic process", "biosynthetic process", among others. The next step is evaluating the presence of SNPs within DEGs that may act as a potential biomarker to favor the selection of more herbivore diet-tolerant fish strains.

FONDECYT 11170847

High Fat Diet Induce Hepatic Hypersensitivity to Glucocorticoids

Francisco Díaz-Castro[1], Jonatan Dassonvalle[1], Camila Donoso[1], Carlos Sepulveda [1], Francisco Pino [1], Miguel Llanos [2], Mario Chiong [3], Rodrigo Troncoso [1,3].
(1) LABINAF, Instituto de Nutrición y Tecnología de los Alimentos, Universidad de Chile, Santiago , CL
(2) Laboratorio de Nutrición y Regulación Metabólica , Instituto de Nutrición y Tecnología de los Alimentos, Universidad de Chile, Santiago , CL
(3) Advanced Center for Chronic Diseases (ACCDiS), Universidad de Chile, Santiago , CL

Introduction:
The glucocorticoids are steroids hormones that are released in response to stress and have an important role in the regulation of the metabolism homoeostasis. The response to glucocorticoids is not determine just by the concentration but also the differences between individuals in glucocorticoid sensitivity, which is influenced by multiple factors like obesity.

Objectives:
The aim of this work is to study the effect of high fat diet in the response to glucocorticoids.

Materials & Methods:
Mice C57BL/6 wild type was separate in two groups, feed by 3 months with a Low-Fat Diet (LFD, 10% fat) or High Fat Diet (HFD, 60% fat), after were injected with a single dose of dexamethasone (DEX, 2 g/kg), after 8 h were sacrificed. The mRNA expression of GRa, GRb, 11b-HSD1, FKBP5, PDK4, PCK1 and G6Pase were assessed by real-time qPCR. The protein levels of total GR, p-GR^{211}, FKBP5, G6Pasa and PCK1 were assessed by western blot.

Results:
In response to dexamethasone the mRNA expression of FKBP51, PDK4, PCK1, G6Pase were higher in HFD in comparation to LFD. Also were increase G6Pase and PCK1 at protein levels. We also evaluate the changes in the protein involved in the regulation of glucocorticoid response. Our results show that phosphorylation of GR does not changes in response to DEX, however the mRNA expression 11b-HSD1 is higher in the mice fed with HFD.

Conclusion:
HFD induces a hepatic hypersensitivity to glucocorticoids that suggest that the metabolic effect of glucocorticoid is increased in subject with obesity.

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Metal specificity on the activity of self-assembled catalytic amyloids

Eva Durán-Meza[1], Claudio Castillo-Cáceres[1], Esteban Nova[2], Octavio Monasterio[1], Rodrigo Diaz-Espinoza[2].
(1) Biología, Ciencias, Universidad de Chile, Santiago, CL
(2) Biología, Química y Biología, Universidad de Santiago de Chile, Santiago, CL

Amyloids are aggregated polypeptides of regular structure stabilized by intermolecular hydrogen bonds. Their involvement in biology is classically associated to neuropathologies. However, many studies have shown that their unique features are exploited by nature for different physiological roles. Moreover, recent works demonstrated that amyloids become catalytic when bound to divalent metals, forming metal-amyloid complexes. The firstly developed catalytically active amyloid-metal complex used a peptide (IHIHIQI) that in presence of zinc (Zn^{2+}) forms active Zn^{2+}-amyloid complexes displaying an esterase activity. Our group also developed a novel catalytic amyloid with a sequence resembling the active site of a nucleotidyltransferase (SDIDVFI). The self-assembly of this peptide into amyloids is strictly metal-dependent and in presence of manganese (Mn^{2+}), the Mn^{2+}-amyloid complex displays an ATPase-like activity. In this work, we addressed the question whether the observed activity is strictly dependent on the metal bound. We performed aggregation experiments with three different metals followed by structural characterization of the amyloids. The results showed that all three metals independently trigger the self-assembly of peptide SDIDVFI into canonical amyloid structures. However, the observed ATPase-like activity is only achieved by the Mn^{2+}-amyloid complex. To confirm our results, we studied the metal specificity on the esterase activity of peptide IHIHIQI and showed that only the Zn^{2+}-amyloid complex is the active species. Additional experiments showed that keeping the active metal and interchanging the peptide abolished the observed activities. Overall, our results demonstrate that the activities of catalytic amyloids appear to depend on both the metal bound and the coordinating residues.

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Using trees to explore the role of the circadian clock in salt- and drought stress

Cristian Ibáñez[1], Alexander Vergara Robles[2], **Eriksson Maria E.[3]**.
(1) Departamento de Biología. Facultad de Ciencias. Universidad de La Serena. La Serena. Chile
(2) Department of Forest Genetics and Plant Physiology, Umeå Plant Science Centre, Swedish University of Agricultural Sciences, 901 83 Umeå , Sweden
(3) Department of Plant Physiology, Umeå Plant Science Centre, Umeå University, 901 87 Umeå, Sweden

Plants adapt growth to their local environmental conditions. Climatic factors such as low precipitation and saline soils significantly compromise their productivity. Deterioration of soils will be dramatically accelerated during this century and there is a need to understand the genetic and molecular factors which are adaptive and could be used to improve growth of crop plants to dry and saline conditions. The circadian clock has evolved to allow anticipation of regular environmental changes to optimize daily and seasonal timing of metabolism and physiological processes, including photosynthesis and stress mitigation. In this study we used Populus trees to investigate drought and salt stress in wild-type (WT) and trees with a perturbation in clock function. We challenged trees with an increasing NaCl or mannitol (osmotic stress) during a week of treatment, to test their responses. Trees obtained NaCl or mannitol (maximal concentration of 150 and 300 mM respectively) as compared to water treated controls. We studied growth, stress responses, metabolites and gene expression. We found that Populus trees were coping well with salt and drought stress within this time frame. We focused on roots and found that the more significant changes occurred after three days, with metabolome changes being the most apparent. Our results will inform further studies of salt stress in Populus with a possible future use in informing the design of next generation crops.

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Frequency of proprotein convertase subtilisin/kexin type 9 (PCSK9) 23968a>g genetic variant in healthy individuals of the region of antofagasta

Hugo Ramirez[3], Galvez Anita S[3], Luis Salazar[1], Alexis Kalergis[2], Jorge Escobar[3].
(1) Departamento de Ciencias Básicas, Facultad de Medicina, Universidad de la Frontera, Temuco, CL
(2) Instituto Milenio de Inmunologia e Inmunoterapia, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, CL
(3) Departamento Biomédico, Facultad de Ciencias de la Salud, Universidad De Antofagasta, Antofagasta, CL

Genetic marker studies are relevant to evaluate the association among allelic variant and susceptibility to develop non-contagious diseases. Identification of allelic variants may allow for early prevention of the development of cardiovascular pathologies, such as the polymorphic PCSK9 gene that promote lysosomal degradation of LDLR. The rs505151 PCSK9 variant is a gain of function mutation resulting in accumulation of processed PCSK9 along with increased LDL levels. The main goal of this study was to evaluate genotypic distribution and allelic frequency of rs505151 PCSK9 genetic variant and the effects on lipid levels in individuals from the city of Antofagasta. This study evaluated 178 healthy adult individuals of both gender. Genomic DNA was extracted from peripheral blood leukocytes through saline precipitation method. Genetic variant was determined by real-time PCR. Plasma was processed to quantitate total cholesterol (TC), triglycerides (TG), HDL and LDL. Lipid profiles were determined by colorimetric enzyme tests. Anthropometric and clinical characteristics such as BMI and blood pressure were normal. Genotypic distribution was AA: 93%; AG: 7% and GG: 0%. Allelic frequencies were A: 0.97 and G: 0.03, consistent with Hardy-Weinberg. Results showed a decrease in TC and TG in male carrying minor allele (G), by the contrary, an increase in TC was observed in women carrying same allele. Heterozygous female group showed higher values of TC, TG, HDL-C and LDL-C compared to male group.It is required to increase the sample size and conduct a case-control study to determine the potential impact of this genetic variant on our population.

Programa Semillero de Investigacion. Vicerrectoria de Investigacion, Innovacion y Postgrado. Universidad de Antofagasta.

The dominant negative ΔTCF4 transcription factor prevents genomic instability events induced by the activation of the Wnt/β-catenin signaling

Espinoza Francisca A [1], Ugarte Giorgia D[1], Verdugo Daniela A[1], De Ferrari Giancarlo V[1].
(1) Instituto de Ciencias Biomédicas, Facultad de Medicina y Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago, CL

Genomic instability is a hallmark of cancer and a number of neurological disorders and evidence has accumulated suggesting that elevated transcriptional rates increase DNA damage. We described earlier that sustained activation of Wnt/β-catenin signaling induces a common chromosome translocation in hematopoietic precursor cells. Here we examined the function of the dominant negative ΔTCF4 transcription factor, which lacks the β-catenin binding domain and is unable to activate Wnt/β-catenin mediated transcription, in the generation of genomic instability events induced by the signaling cascade. Our results show that sustained activation of Wnt/β-catenin signaling in HEK293 cells, by using a constitutively active β-catenin S33Y (10, 20 and 40 ng, 24 - 48h), induced a significant increase in DNA breaks, as measured by single-cell electrophoresis assays and γ-H2AX immunofluorescence staining. Notably, a significant decrease in DNA breaks was observed when these cells were co-transfected with increasing doses of the ΔTCF4 (24-48 h) construct. Our results suggest that the TCF4 (TCF7L2) transcription factor is an interesting target for designing new therapies for a broad spectrum of pathologies.

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Differential effects of Palmitic and Oleic acid on morphology and interaction between lipid droplet and mitochondria in HepG2 human cells

Andrea Eynaudi[1], Camila Donoso[2], Valentina Parra [3,4], Rodrigo Troncoso [2,4].
(1) Instituto de Nutrición y Tecnología de los alimentos , Instituto de Nutrición y Tecnología de los alimentos , Universidad de Chile, Santiago, CL
(2) Laboratorio de Investigación en Nutricion y Actividad Fisica , Instituto de Nutricion y Tecnologia de los Alimentos , Universidad de Chile, Santiago , CL
(3) Departamento de Bioquímica y Biología Molecular, Facultad de Química y Farmacia, Universidad de Chile, Santiago, CL
(4) Advanced Center for Chronic Diseases (ACCDiS), Universidad de Chile, Santiago , CL

Introduction:
Fatty liver disease is characterized by the accumulation of triglycerides through dynamic structures called lipid droplets (LDs). It is described that palmitic acid (PA) as oleic acid (OA) produces steatosis, but only treatment with PA produces mitochondrial dysfunction and insulin resistance with progression to liver damage. To date, is unknown how these fatty acids can differentially affect the LD morphology and their interaction with mitochondria; the most relevant organelle in charge of energy homeostasis, as well as for the proper functionality of the hepatocyte.

Objectives:
To evaluate the effect of lipid overload by the treatment of PA or OA on the LD morphology and their physical interaction between LD-mitochondria in HepG2 cells.

Methodology:
Hepatocytes were exposed for 2, 6, 18 and 24 h to 200 µM PA or 200 µM OA conjugated to bovine serum albumin (BSA). Then, cells were incubated with BODIPY and MitoTracker Orange for LD and mitochondria staining respectively. Hepatocytes, were later fixed and mounted for Confocal microscopy imaging and image analysis (LD number and volume and colocalization with mitochondria) using ImageJ, NIH.

Results:
OA treatment showed a greater number and volume of LDs. In relation to Mitochondria-LD colocalization, OA showed a higher colocalization in comparison to PA treatment and control hepatocytes (BSA).

Conclusion:
Our results show that PA promotes the formation of smaller and fewer LDs, with a reduced physical interaction with mitochondria in comparison with OA. These results suggest that the LD morphology and interaction with mitochondria can be determinant in PA-induced mitochondrial dysfunction.

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Phyllanthus emblica extract induces oncosis-like cell death and inhibits cell migration of HeLa cells

Alejandro Farías[1], Jean Pierre Köpplin[1], Génesis Fuentes[1], Sergio Bustamante[1], Ariel Castro[1], Violeta Morín[1].
(1) Bioquímica y Biología Molecular, Ciencias Biológicas, Universidad de Concepción, Concepción, CL

Cancer treatment implies the use of extremely toxic chemotherapy drugs that involve a wide range of side effects. For this reason, it has arisen the need for searching new and more effective therapies. The fruit of Phyllanthus emblica (Amalaki) has been employed as a natural treatment for various illnesses including cancer. Our studies focused on the determination of antitumour effects of Amalaki. Assays with Methyl Violet showed that Amalaki produced a 70% decrease on HeLa cells viability. In addition, relevant morphological changes such as the formation of a large vacuole and loss of nuclear morphology were observed. These results, altogether with Western Blot analysis suggest a process of cellular death known as Oncosis. On the other hand, wound healing assays showed that HeLa cells treated with Amalaki decrease their migration capacity due to the increase in E-cadherin expression. To determine Amalaki´s selectivity, we also analyzed the effect of the extract on the colon epithelium 841 CoN cell line; the cellular viability of this cell line decreased in a lower proportion than the cancerous line. Besides, cell morphology was not affected by Amalaki treatments. BrDu proliferation assays showed that there is a decrease in the replication capacity of 841 CoN cells; conversely, no replication was observed in the cancerous cell line due to nuclear damage. These particular results show that Amalaki has antitumour properties, but additional studies are necessary to implement it as a possible cancer treatment.

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Lower body weight in rats under hypobaric hypoxia exposure would lead to lesser right ventricle hypertrophy and more AMPK activation

Karen Flores[1], Patricia Siques[1], Julio Brito [1], Karem Arriaza[1], Mº Rosario López [2], Ángel Luis López De Pablo [2], Silvia Arribas[2].
(1) Institute of Health Studies, Universidad Arturo Prat, Iquique, CL
(2) Department of Physiology, Universidad Autónoma de Madrid, Madrid, ES

Background:
The chronic hypoxia (CH) and long-term chronic intermittent hypoxia (CIH) exposure leads to right ventricular hypertrophy (RVH). Weight loss would be an effective intervention to improve cardiac function and energy metabolism in cardiac hypertrophy, and plays an important role in the cardioprotection through AMPK activation. The aim was to determine the body weight influence in RVH, AMPK and related variables comparing rats exposed to both hypoxic conditions.

Methods:
Sixty male adult rats according to their diet were separated in two group (n=30): caloric restriction (CR) (10g daily) and ad libitum (AL). Both groups were randomly assigned to 3 groups: NX (normoxic; n=10), CIH (2 days hypoxia/2 days normoxia; n= 10) and CH (n=10). Rats were exposed to simulated hypobaric hypoxia at 4,600m for 30 days. Measurements included; Body weight, hematocrit, plasma insulin and glycemia, RVH, GLUT1, GLUT4, PP2C level, AMPK, p38MAPK, mTOR and eNOS activation in right ventricle.

Results:
A lesser RVH and a higher AMPK activation were found in CR groups exposed to hypobaric hypoxia ($p<0.05$). Also, there was a decreased glycemia and serum insulin, and increased insulin sensitivity ($p<0.05$). An increased p38MAPK activation in all hypoxic and CR and AL ($p<0.05$) was found. Interestingly, PP2C and mTOR only increased in the AL groups ($p<0.05$), while eNOS was decreased ($p<0.05$).

Conclusion:
Maintaining low weight under high altitude-hypoxia, would prevent a major right cardiomyocyte hypertrophy effect. This cardioprotection would probably be due to AMPKactivation. Thus, weight control might be considered as an important tool in clinical field.

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Novel halophilic glucose-6-phosphate dehydrogenases form archaea belongs to the SDR superfamily

Nicolás Fuentes-Ugarte[1], Pablo Maturana[1], Ricardo Cabrera [1], Víctor Castro-Fernández[1], Victoria Guixé[1].
(1) Laboratorio de Bioquímica y Biología molecular, Facultad de Ciencias, Universidad de Chile, Santiago, CL

The first report on the operation of the oxidative pentose-phosphate pathway in archaea involved the characterization of a novel glucose-6-phosphate dehydrogenase (G6PDH) in the *Halobacteria Haloferax volcanii* (HvG6PDH). Organisms from this class thrive in environments with high salt concentrations being their proteins adapted by reducing its hydrophobic core and increasing, their acid residues proportion. The HvG6PDH enzyme is not related to bacterial and eukaryal enzymes but belongs to the short-chain dehydrogenases/reductases (SDR) superfamily which display great functional diversity. The archaeal G6PDH phylogeny shows that these proteins are exclusively present in the *Halobacteria* class. Through evolutionary profiling and homology modeling we showed that in addition to the presence of all the previously described SDR motifs, a conserved triad (NLT) occurs in a loop near to the active site that interacts with the glucose-6-phosphate substrate. Bioinformatic analysis shows that HvG6PDH presents the canonical traits for halophilic proteins, that is a high content of acidic residues (Glu+Asp, 18.4%) and a low content of Lys (1.9%). Enzyme activity measurements indicates that HvG6PDH has its optimal activity at salt concentrations over 2 M. Influence of the NaCl and KCl concentrations on the kinetic parameters was assessed. Both salts increase mainly the V*max* with a slightly decrease in the K*m* for glucose-6P. The enzyme was also able to oxidize glucose, beside glucose-6P, with a catalytic efficiency of approximately 300-fold higher for the later. These results provide valuable information for the understanding of the SDR superfamily evolution in halophilic archaea and catalysis at high salt concentrations.

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Technical and educational validation of a mobile phone colorimeter

María José Gallardo[1], Victor Gomez[1], Camila Godoy[1,2], Fernanda Zamorano[1,2].
(1) Lab4Chemistry, Lab4U, Santiago, CL
(2) Facultad de Química y Biología, Universidad de Santiago, Santiago, CL

The purpose of the colorimeter is to provide the general population with an application of easy use and from which images of the solutions are used to evaluate the color of these test solutions. Optical-chemical analyzes are the most common methods for determining the concentration of a solute in a solution, based on the ability of compounds to absorb light energy. However, all these techniques require large and expensive equipment, so that the analysis of samples must be performed within a laboratory facility. Furthermore, the digital colorimeter is an economic and easy technical solution compared to the use of known spectrophotometric equipment and has the additional advantage that requires no external devices. The system calculates the concentration of a solute in a solution from colored imaging which includes a calibration curve. A set of solutions of known concentrations and the unknow sample must be placed in test tubes to take a picture. The captured image requires internal processing by the application for obtaining the color values, for which executes a pre-processing of the image, where an approximation of the area of each sample is obtained and detects the image in a matrix of pixels in the RGB color space, which applies a Gaussian interpolation filter to remove background noise. This application was implemented for teaching basic concepts of chemical analysis in educational establishments. The digital colorimeter provides a solution for educational classrooms (schools and universities) requiring practical teaching cases or those requiring analyzing samples on site, outside of the laboratory facilities.

Proyecto CORFO 17ITE2-88850

Surviving stress: Identification of genes involved in hydric stress response in the Antarctic plant *Colobanthus quitensis*

Carolina Galleguillos[1,2], Rasme Hereme[1,2].
(1) Doctorado en Ciencias mención Ing. Genética Vegetal, Instituto de Ciencias Biológicas, Universidad de Talca, Talca, CL
(2) Ecología y Biodiversidad, Instituto de Ciencias Biológicas, Universidad de Talca, Talca, CL

In Antarctica, water available is scarce, so some authors agree that this is the main limiting resource for plant development. However, in Antarctica it is possible to find two native vascular plants; one of them is *Colobanthus quitensis*, which has served as a study model due to its ability to survive under such stressful conditions as water stress. On the other hand, DREB/CBF transcription factors have been widely studied due to their important participation in the response to abiotic stress in plants, mainly water stress, by inducing genes involved in stress tolerance (dehydrins) and at the same time they are capable of inducing the transcription of genes involved in development and flowering processes, such as Aux / IAA repressors. That is why, the objective of this research is to evaluate the relative expression of four *DREB/CBF*, five dehydrins and six *Aux/IAA*, in order to define the genes that are activated in response to water stress, as well as the measurement of physiological and biochemical parameters stress markers. For this, individuals from the Shetlands Islands (62° Lat. S) and Antarctic Peninsula (68° Lat. S) were evaluated. The results show significant differences in relative expression between different genes. And, based on the stress indicator parameters, the population of the peninsula proved to be the most sensitive to this condition, so it can be suggested that individuals from this population are not constantly subjected to severe stress conditions compared to those from latitude 62° S.
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GDF11 prevents cardiomyocyte hypertrophy by maintaining ER-mitochondria communication

Valeria Moreno[1], Mayarling-Francisca Troncoso[1], Camila López-Crisosto[1], Mariana Cifuentes[1,2], Sergio Lavandero[1,3].
(1) Advanced Center for Chronic Disease, Universidad de Chile, CL
(2) Institute of Nutrition and Food technology, University of Chile, CL
(3) Department of Internal Medicine, Southwestern Medical Center, University of Texas, Dallas, Texas, US

Introduction:
Cardiomyocyte hypertrophy is characterized by increases in cell size, contractile protein synthesis and stimulation of fetal gene expression in a response to chronic stress. Our previous work showed that the hypertrophic agent norepinephrine (NE) induced the loss of ER-mitochondria communication. Growth/Differentiation Factor 11 (GDF11) belongs to TGFβ family and has shown controversial effect on cardiac hypertrophy. We hypothesized that GDF11 is an anti-hypertrophic agonist that prevents the loss of communication RS-mitochondria in cultured cardiomyocytes treated with NE.

Methods:
Neonatal rat ventricular myocytes (NRVM) were pretreated with or without GDF11 10 nM for 6 h and then incubated with NE 10 µM for 48 h. Cardiomyocyte area and perimeter were determined in cells stained with rhodamine-phalloidin and epifluorescence microscopy. Protein and mRNA levels for ANP (hypertrophy biomarkers) were assessed by Western blot and qPCR, respectively. ER-mitochondria proximity was evaluated by immunofluorescence microscopy using antibodies against Grp75 and KDEL. To study functional communication between both organelles, mitochondrial and cytoplasmic Ca2+ levels were determined using Ca2+ using Rhod 2AM and FURA 2AM. The activation of calcineurin signaling pathway was determined by RCAN protein levels.

Result:
GDF11 prevented: a) NE-dependent increases in cell area and perimeter and in protein and mRNA levels of ANP. B) NE-induced loss of contact sites and functional communication between ER and mitochondria and the activation of calcineurin pathway as well.

Conclusion: Collectively these results suggest that GDF11 prevents NE-dependent cardiomyocyte hypertrophy by maintaining ER-mitochondria communication.

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Evaluation of physiological and metabolite traits related to productivity and water stress tolerance in wheat under field conditions

Miguel Garriga[1], Ana María Méndez-Espinoza[1], Félix Estrada[1], Sebastián Romero-Bravo[2], Angie L. Gámez[3], Iker Aranjuelo[3], Alejandro Del Pozo[1].
(1) Centro de Mejoramiento Genético y Fenómica Vegetal, Facultad de Ciencias Agrarias, Universidad de Talca, Talca, CL
(2) Facultad de Ciencias Agrarias y Forestales, Universidad Católica del Maule, CL
(3) Instituto de Agrobiotecnología (IdAB) , ES

The identification of physiological traits related to performance and tolerance to water stress is a major goal of wheat breeders to improve the selection of genotypes in breeding programs. A set of 12 genotypes of wheat (*Triticum aestivum* L.) were tested under irrigated (FI) and water stress (WS) in field conditions. Grain yield (GY) and its components were evaluated, as well as several physiological traits, leaf water potential (WP), gas exchange, modulated chlorophyll fluorescence, leaf content of pigments, water-soluble carbohydrates, and amino acids. Principal component analysis (PCA) and Pearson regression analysis were performed to establish relationships between the productive traits and physiological and metabolite traits, and to identify traits related to the plant response under water stress. PCA analysis performed independently for physiological and metabolite traits explained approximately 70% of the observed variability. The first component (PC1) was associated with WP, gas exchange traits (An, Gs, E), non-photochemical fluorescence traits (Y(NPQ), Y(NO), qN), and metabolite content of sucrose (Suc) and several amino acids. High and positivity correlations ($r \geq 0.8$) between GY and WP, An, Gs, E, Y(NO) and Suc content were observed. High and negative correlations ($r \leq 0.8$) took place between GY and Y(NPQ), Y(NO), qN and amino acid contents (Pro, Leu, Ile, Phe, Tyr). Although more studies are needed, some of these traits could be incorporated into field phenotyping platforms for a more efficient selection of wheat genotypes with improved tolerance to water stress.

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Quantitative determination of intrinsic propensities of natural DNA sequences to assemble as nucleosomes

Gidi Cristián A [1], David Muñoz[2], Sylvain Marcellini[2], Gutiérrez José L[1].
(1) Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad De Concepción, Concepción, CL
(2) Departamento de Biología Celular, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, CL

Histones bind DNA in a non-sequence dependent manner. However, it has been observed that some DNA sequences show differences in their propensity to form nucleosomes. This variation in affinity has been mainly attributed to the stiffness of the DNA chain, which could increase or decrease the energy needed for nucleosome formation. In vivo studies have found strongly positioned nucleosomes in defined genomic regions, while other regions are depleted of nucleosomes. This pattern could rely, at least in part, on the aforementioned properties of DNA sequences. To quantitatively determine the propensity to assemble as nucleosomes of selected natural DNA sequences, we used an experimental approach called “competitive reconstitution”. A series of sequences belonging to an enhancer region from *Xenopus tropicalis* and to the proximal promoter region of the rat osteocalcin gene were analyzed. We obtained 32P-labeled 147bp fragments by PCR and reconstituted them as mononucleosomes. The reconstituted mononucleosomes were subjected to electrophoresis in native polyacrylamide gels. The ratio of non-nucleosomal DNA to nucleosomal DNA was used for equilibrium constant and ΔG calculations. Our results show that each genomic region analyzed harbors sequences displaying high and low relative propensity to assemble as nucleosomes. These results will contribute to a better understanding of the nucleosome excluding properties of DNA on specific genomic regions that are potential subjects for gene regulation at the chromatin level, which is crucial for our understanding of chromatin dynamics and gene expression.

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Molecular Crosstalk Between Down Syndrome and Alzheimer´s disease: A Bioinformatics Approach

Wileidy Gómez[1,2], Carla Arias-Duran[1], Vinicius Maracaja-Coutinho[1], Melisa Nassif[2], Valentina Parra[1].
(1) Advanced Center for Chronic Diseases , Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, CL
(2) Center for Integrative Biology (CIB), Ciencias, Universidad Mayor, Santiago, CL

Introduction:
Down syndrome (SD) is the most common chromosomal disorder, and adults individuals affected with this condition present a high rate of Alzheimer´s disease (AD), the most prevalent neurodegenerative disease in the world. DS brain present early neuropathological hallmarks of AD, including accumulation of amyloid-β and tau hyperphosphorylation. Common intracellular mechanisms associated with these hallmarks include the trisomy of APP gene, present in chromosome 21, and the dysfunction of autophagic and lysosomal pathways. However, limited information is available regarding genes other than APP related to premature AD development in DS.

Methods:
Using bioinformatic tools for the meta-analysis of public data, we determined shared metabolic pathways and novel co-altered expressed genes in DS and AD, and using the RT-qPCR technique we were able to validate the expression of some target genes in trisomic induced pluripotent stem cell (iPSC 3S) from people with DS.

Results:
We found that 24 genes were upregulated in both conditions, including CITED2 along with the finding that one of the main co-affected primary metabolic pathways in DS and AD is the process of selective mitochondrial autophagy or mitophagy. Besides, we found a higher level of CITED2 transcripts in iPSC 3S than in 2S.

Conclusion:
Our results suggest mitochondrial quality control as a common metabolic pathway altered in both conditions, DS and AD. We propose that the identification of new genes (such as CITED2) that participate in the mitophagy process and the manipulation of their expression can reduce the damage caused by oxidative stress linked to AD.

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Theoretical and statistic study of the thermodynamics parameters involved in the activation process of TRPV1

Felipe Gómez-Alvear[1,2], Ignacio Diaz-Franulic[1], Daniel R. Aguayo-Villegas[2], Ramón Latorre[2], Fernando D. González-Nilo[2].
(1) Instituto Milenio Centro Interdisciplinario de Neurociencia de Valparaíso , Facultad de Ciencias, Universidad de Valparaíso, Valparaíso , CL
(2) Center for Bioinformatics & Integrative Biology, Ciencias de la Vida, Universidad Andrés Bello, Santiago, CL

The knowledge of the TRPV1 structure thought CryoEM under different conditions of activation (open *PDB id: 3J5Q*and closed *PDB id: 3J5P*) provides an excellent framework to identify the thermodynamic parameters that drive the channel activation. In this work, we describe the difference of free energy profile between the open and closed state of TRPV1. To illustrate the process of the activation channel, we used the Adaptive Biasing Force (ABF) method, implemented in NAMD, to calculate the potential mean force profile. The challenge of this kind massive calculations is define the reaction coordinate of the conformational change that occurs during the activation of the channel. To solve this problem, we use the RMSD function as a collective variable to define the best reaction coordinate that we can calculate with ABF. As a complement of PMF calculation, we use ANOVA analysis of SASA through the trajectory to calculate de entropy contribution between both states (open and closed). The results of this study are consistent with our experimental measurements for the wild type and mutations designed to disturb the heat sensitivity. Thus, our theoretical and experimental results contribute to a better understanding of the thermodynamics parameters that govern the activation process of TRPV1 at the molecular level.

Instituto Milenio Centro Interdisciplinario de Neurociencia Valparaíso, ICM-MINECON P09-022-F. Fondecyt Regular 1170733. Center for Bioinformatics and Integrative Biology, Facultad Ciencias de la Vida, Universidad Andrés Bello.

Toxic effects of retene in human colon cells

Matías González[1], Ariel Castro[2], Violeta Morín[3].
(1) Laboratorio de Proteasas, Ciencias Biológicas, Universidad de Concepción, Concepción, CL
(2) Laboratorio de transducción de señales y cáncer, Ciencias Biológicas, Universidad de Concepción, Concepción, CL
(3) Laboratorio de Proteasas, Ciencias Biológicas, Universidad de Concepción, Concepción, CL

In Chile, the forestry industry is an important productivity sector. The cellulose is the most important product made by this industry. This productivity process generates a considerable amount of residual hydrocarbon compounds like phytohormones, steroles, benzo[a]pyrene and retene. The effect of the latter on human health has not been well-studied. We hypothesized that the retene has an impact in the viability of human colon cells and that it is degraded by the cells through the common xenobiotics degradation pathway. The effect of retene on viability of the human colon CCD-814 cell line was measured by crystal violet staining. To approach the interaction between retene and biodegradation enzymes, we performed an *in silico* assay, using the structure of retene and cytochrome p450 for molecular docking. In addition, we analyzed the expression levels of pollution marker genes at different exposure times through RT-PCR. The viability assay showed that retene produced a small decline in the cells number. The LC10 was 2.407µM and the LC20 3.1922 µM. Moreover the molecular docking showed that there are energetically favored retene poses in the active site of CYP1A1 and CYP1B1, being the affinity values -13.6 kcal/mol and -3.8 kcal/mol, respectively. Finally, according to the RT-PCR results, long retene exposure time increased the expression levels of CYPs and aryl hydrocarbon receptor. Our studies suggest that CYP1A1 and CYP1B1 can metabolize compounds like retene and that the human colon cells are a good model for environmental toxicology assays.

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DNA binding selectivity of known marboxes by bipartite HTH proteins MarA, Rob and two artificial but related variants

Constanza Guerra[1], Francisco Melo[1].
(1) Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile, Santiago, CL

MarA and Rob are DNA binding proteins that belong to the AraC/XylS family of bipartite Helix-Turn-Helix (HTH) transcription factors. In the case of MarA protein, a total of 33 target DNA binding sites, called marboxes, have been described. Many of these marboxes are also recognized by Rob protein. In this work, we assessed the DNA binding selectivity of MarA and Rob proteins for these 33 known marboxes by an experimental setup that consisted of *in vitro* binding, electrophoresis separation of bound and unbound complexes and next generation sequencing of bound DNA variants. The results show that only a few marboxes (5-13 out of 33) are preferentially bound by these proteins at a nanomolar scale. These marboxes include *marRAB*, *ybaO*, *micF*, *purA*, and *rob*. The marboxes binding preference is also differential between MarA and Rob proteins. In general terms, Rob protein is more selective than MarA protein. The addition of the regulatory domain of Rob to MarA protein (an artificial chimeric fusion protein) causes an increased selectivity towards *ybaO*, *marRAB* and *micF* marboxes. The deletion of the regulatory domain of Rob (Rob-DNA-binding domain only, defined as Rob-DBD) shows a selectivity pattern more similar to Rob (5-7 marboxes preferentially bound) than MarA (10-13 marboxes preferentially bound). These results suggest that the position weight matrices (PWMs) currently in use for these transcription factors and derived from all 33 known marboxes may not represent an accurate model of DNA binding specificity for MarA and Rob proteins.

Two pathways, one stress: Endophytic fungi and their role in the induction of transcription factors under drought stress in *Colobanthus quitensis*

Rasme Hereme[1,2], Andrea Barrera[1,2], Carolina Galleguillos[1,2], Patricio Ramos[2,3].
(1) Doctorado en Ciencias, mención Ingeniería Genética Vegetal, Universidad de Talca, Talca, CL
(2) Ecología y Biodiversidad, Instituto de Ciencias Biológicas, Universidad de Talca, Talca, CL
(3) Nucleo cientifico multidisciplinario, Direccion de Investigación, Universidad de Talca, Talca, CL

Antarctic is a unique place for the study of adaptive mechanisms in plants, due to stressful conditions such as water deficit. In addition, in extreme environments, the presence of endophytes in plants becomes relevant, since it allows a better capacity to tolerate stressful conditions. At the molecular level, plants respond to hydric stress through dependent or independent pathway of Abscisic Acid (ABA), being key in the induction of these pathways transcription factors (TFs). However, there is no information about the role of endophytes (E) on the expression of TFs involved in the responses under water stress conditions in the Antarctic plant *Colobanthus quitensis*, as well as the role of the presence of endophytic fungi would have in the induction of molecular signals in response to stress. Here, we evaluate the induction of TFs mediated drought-responsive genes of dependent (*CqABF* and *CqMYC2*) and independent (*CqDREB1* and *CqDREB2*) pathway of ABA, in plants with endophytic fungi (E+) and without endophytic fungi (E-) of *C. quitensis*, as well as biochemical and physiological responses, under condition of hydric stress, which represents 50% of water availability respect to the current availability. The results show that the induction of TFs was mediated by the presence of endophytes, however, there was no observed a prevalence of one pathway over the other; besides E+ plants show a better osmoregulation and less oxidative damage compared to E-. These results suggest that presence of fungal endophytes result in a key strategy for tolerate water deficit of Antarctic plants.

Angiotensin- (1-9) prevents lipotoxic stress-induced hypertrophy in cultured cardiomyocytes

Carolina Hernandez-Fuentes[1], Felipe Muñoz[1], Valentina Parra[1], Sergio Lavandero[1,2].
(1) Advanced Center for Chronic Diseases (ACCDiS), Faculty of Chemical and Pharmaceutical Sciences & Faculty of Medicine, Universidad de Chile, Santiago, CL
(2) Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, Texas, US

Introduction.
The classical renin-angiotensin (RAS) system has a counter-regulator axis known as alternative RAS that exerts cardioprotective action on cardiomyocytes properties. Angiotensin-(1-9) (Ang-(1-9)), one of the main effectors of the alternative RAS, prevents angiotensin II and norepinephrine-induced cardiac hypertrophy. This last cellular process is characterized by increases in cardiomyocyte size, sarcomere number, contractile protein content (i.e. beta-myosin heavy chain) and by the reexpression of fetal gene program (i.e. ANF). Interestingly, excessive accumulation of lipids (lipotoxicity) in the heart also promotes morphological and metabolic changes in cardiomyocytes, including the development of cardiac hypertrophy. In the present study, we evaluate whether Ang-(1-9) prevents the effect of lipotoxicity on cardiac hypertrophy.

Methods & Results.
Cultured neonatal ventricular myocytes (NRVM) were treated with myristate (500 µM) or palmitate (328 µM) for 24 h with or without pre-incubation with Ang(1-9) (100 µM) for 6 h. Then, protein levels of b-MHC and ANF were determined and used as hypertrophic markers. Our results showed that palmitate, but not myristate, significantly increases b-MHC levels, being this effect prevented with Ang-(1-9). Both palmitate and myristate attenuated insulin-dependent activation of Akt, suggesting a state of insulin-signaling desensitization.

Conclusions:
Palmitate, but not myristate, promotes morphological and metabolic changes in cardiomyocytes. Ang-(1-9) prevents lipotoxicity-induced cardiomyocyte hypertrophy.

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Interleukin 6 promotes migration but not proliferation of vascular smooth muscle cells

Tomás Hernandez-Díaz[1], Paulina Villar-Fincheira[1], Ignacio Norambuena-Soto[1], Nicole Cancino-Arenas[1], Fernanda Sanhueza-Olivares[1], Mario Chiong[1].
(1) ACCDiS. Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, CL

Introduction:
Vascular smooth muscle cells (VSMC) are the main component of the tunica media in the arteries. They possess phenotypic plasticity changing from a differentiated to a dedifferentiated state. Dedifferentiated VSMCs are characterized by increased proliferation and migration. This phenotype is associated with vascular remodeling induced by exercise. Interleukin-6 (IL-6) is a myokine released by skeletal muscle after physical activity. Little is known about the effects of IL-6 in VSMCs phenotype.

Hypothesis:
IL-6 induces VSMC dedifferentiation.

Methods:
Vascular smooth muscle cell line A7r5, were treated with IL-6. Platelet-derived growth factor-BB (PDGF-BB) was used as positive control for dedifferentiation. Cell migration was evaluated by wound healing and transwell assays. Proliferation was assayed by MTT. VSMC contractile proteins α-SMA and calponin, were assessed by western blotting.

Results:
IL-6 induced VSMC migration assessed by wound healing and transwell assays. IL-6 induces VSMC proliferation. IL-6 did not change α-SMA and calponin protein levels.

Conclusion:
IL-6 induces VSMC migration and proliferation without change in α-SMA levels. Our findings may help to elucidate how sport decreases the likelihood of cardiovascular disease, and in particular, atherosclerosis.

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Photosynthetic oxygen generation under physiological conditions for the treatment of tissue ischemia

Christopher Holmes[1].
(1) Instituto de Ingeniería Biológica y Médica, Ingenieria, Pontificia Universidad Católica de Chile

Several groups have suggested the use of photosynthesis as a novel approach for oxygenating hypoxic tissues, providing a controllable source of oxygen that is independent of the vasculature of the organism. Yet, human physiology evolved separately from plants and does not have the necessary physiological requirements needed to sustain photosynthesis. Thus, arises the question of how photosynthetic cells will behave in human physiological conditions, which is a key question that needs to be answered for the development of effective photosynthetic therapies that reach clinical settings. Thus, to answer this question in this work we characterized the photosynthetic performance and viability of the model organism *Chlamydomonas reinhardtii* under cell culture conditions simulating human physiology. In order to confirm the potential physiological relevance of the generated oxygen, *C. reinhardtii* was co-cultured with different human cells, where viability and cell death was quantified. Furthermore, we analyzed the capability of these microalgae for re-oxygenating hypoxic cell cultures through western blot analysis of hypoxia markers. We observed excellent biocompatibility among both cell types with minimal viability loss up to 24 hours of co-culture. Moreover, the microalgae were able to produce enough oxygen to effectively negate the apparition of hypoxia markers. The obtained results provide evidence of biocompatibility between *C. reinhardtii* and human cells under physiological conditions and strongly supports the feasibility of photosynthetic cell therapy for the treatment of pathophysiological conditions related to tissue ischemia.

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Acrylonitrile derivatives as potential inhibitors of NADPH oxidase 2 (NOX2)

Treuer Adriana V. [1,2,3], Erwin Hovelmeyer[1], Roberto Rosales[1], Mario Faundez[4], Gonzalez Daniel R.[3], Ariela Vergara-Jaque[1,5].
(1) Center for Bioinformatics and Molecular Simulation, Universidad de Talca, Talca, CL
(2) Laboratory of Organic Synthesis and Biological Activity, Institute of Chemistry of Natural Resources, Universidad de Talca, Talca, CL
(3) Department of Basic Biomedical Science, Faculty of Health Sciences, Universidad de Talca, Talca, CL
(4) Department of Pharmacy, Laboratory of Pharmacology and Molecular Toxicology, Faculty of Chemistry, Pontificia Universidad Catolica de Chile, Santiago, CL
(5) Millennium Nucleus of Ion Channels-associated Diseases (MiNICAD), CL

The NADPH oxidase isoform 2 (NOX2) system is responsible for reactive oxygen species (ROS) production in neutrophils and has been recognized as a key mediator in inflammatory disorders and several cardiovascular pathologies. The functional NOX2 system involves a set of protein-protein interactions comprising the NOX2 subunit and the proteins p22*phox*, p47*phox*, p67*phox*, p40*phox*, Rac1 and Rac2.A wide variety of compounds have been evaluated to inhibit the production of ROS by targeting proteins of the NOX2 system. Specifically, the binding interface between p22*phox* and p47*phox* have been identified as an important pharmacological target. In the present work, we synthetized heteroaryl-acrylonitrile derivatives and evaluated their function to inhibit NOX2-derived ROS production upon stimulation in neutrophils. Four compounds (i.e. c3c, c3f, c3i, c3n) were found to inhibit superoxide production at comparable levels as those reported for commercial inhibitors (i.e. Apocynin, VAS2870, GSK2795039). Western blot analysis revealed the association of these compounds with p47phox. Based on these findings, we analyzed the binding modes of the synthetic and commercial inhibitors against p47phox by using docking and molecular dynamics simulation. Each complex was evaluated through the MM-GBSA method to estimate its relative binding affinity. In agreement with experimental assays, computational predictions support the association of a lead compound that interacts with p47*phox* in the binding interface with p22*phox*. A group of polar residues in the p47*phox* structure were found to form the binding cavity for the inhibitors. Overall, our study provides a new ROS inhibitor that modulate the activation of the NOX2 system.

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Ethanol extract of *Annona cherimola* seeds showed a selective and apoptotic action against human stomach gastric adenocarcinoma cell line AGS

Johan Macuer-Guzmán[2], Giuliano Bernal[1], Fabiola Jamett-Díaz[3], Sebastián Ramírez-Rivera[1], Cristian Ibáñez[2].
(1) Laboratorio de Biología Molecular y Celular del Cáncer, Departamento de Ciencias Biomédicas, Facultad de Medicina, Universidad Católica del Norte
(2) Departamento de Biología. Facultad de Ciencias. Universidad de La Serena. La Serena. Chile
(3) Departamento de Química. Facultad de Ciencias. Universidad de La Serena. La Serena. Chile

Annona cherimola seeds are considered waste. In this study, we evaluate the antiproliferative activity of ethanol macerate extract (EMCHS) obtained from A. cherimola seeds against the human stomach gastric adenocarcinoma (AGS) cell line and the normal human gastric epithelial cell line (GES-1). Our results showed that EMCHS extract had an IC_{50} of 80.43 µg/mL in AGS cells, and a selectivity index (SI) of 3.5-fold higher than that of the chemical control cisplatin. In addition, the EMCHS extract showed apoptotic activity in AGS cells since 50 µg/mL and. overxpression of PUMA gene in both cells demonstrate that EMCHS activate the apoptotic route. Future studies should be carried out to elucidate anticancer activity of EMCHS in vivo. This work represents the first showing antiproliferative effects of crude extracts obtained from seeds of A. cherimola in AGS cells.Our study revalues the seeds of this species as a potential source of a natural pharmacological product with anticancer properties.

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TGF-β1 down-regulates caveolin-1 expression during cardiac fibroblast differentiation

Danica Jiménez[1], Mónica Villa[1], Ignacio Norambuena-Soto[1], Sergio Lavandero[1,2], Lorena García[1].
(1) Advanced Center of Chronic Diseases (ACCDiS), Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, CL
(2) Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, Texas, US

Introduction:
In order to repair injured tissue, TGF-β1 stimulates cardiac fibroblast differentiation to cardiac myofibroblast as well as the expression of the gene program involved in the development of cardiac fibrosis. In NIH-3T3 cells, the scaffold protein caveolin-1 (CAV-1) negatively regulates TGF-β1 signaling.

Objective:
To study whether TGF-β1 regulates CAV-1 expression in differentiated cardiac fibroblasts.

Methods and results.
Cultured adult rat cardiac fibroblasts were pre-incubated for 30 min with or without the ERK inhibitor PD98059 (10 nM) or TβRI inhibitor (SD208 200 nM) before the treatment with TGF-β1 (0-20 ng/mL for 96 h). CAV-1 and EDA-fibronectin (fibrosis marker) levels were assessed by Western-blot and soluble collagen-1 (COL-1) by Sirius red staining. Differentiated cardiac fibroblast morphology (α-SMA fibers) was evaluated by immunofluorescence microscopy. The results showed TGF-β1 (10 ng/mL for 96 h) stimulates αSMA fibers formation, EDA-fibronectin levels and COL-1 secretion, being all these effects prevented by ERK and TβRI inhibitors. TGF-β1 also stimulated a down regulation of CAV-1 protein levels but independently of ERK and TβRI.

Conclusions.
TGF-β1 regulates negatively CAV-1 levels in differentiated cardiac fibroblasts by Smad/ERK-independent signaling pathway.

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Chymosin immobilization onto eggshell membranes

Eduardo Kessi[1], Víctor Castro Fernández[2], Sonia Vidal[3], José Luis Arias[1].
(1) Departamento de Ciencias Biológicas Animales, Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, CL
(2) Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, CL
(3) Laboratorio de Vacunas Veterinarias, Departamento de Ciencias Biológicas Animales, Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, CL

Enzyme availability and stability may be improved through enzyme immobilization, which provides an excellent base for increasing availability of enzyme to the substrate with greater turnover over extended times. Immobilized enzymes are preferred over their free counterpart due to their prolonged availability that curtails redundant downstream and purification processes. Avian eggshells are biomineralizing systems extensively represented in nature. The innermost layer of an eggshell is composed of two non-mineralized sublayers, the so-called inner and outer shell membranes structured by collagens (types I, V and X) and other proteins and glycoproteins containing lysine-derived cross-links. Mild conditions (i.e. dilute acetic acid treatment) are enough to separate the membranes from the calcified layer providing a very useful and cheap matrix suitable for immobilization procedures. In the present work we describe for the first time the immobilization of recombinant camel chymosin (EC 3.4.23.4) onto glutaraldehyde-derivatized eggshell membranes. Thus, we show the eggshell membrane is an appropriate waste material for immobilizing enzymes suggesting a promising avenue of applications, for example, in the food industry.

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Reduction of oxidative stress in *Arabidopsis thaliana* with saline stress applying extracts obtained from microalgae native from the Tarapacá Region

Gustavo Klapp[1], Alvaro Pimentel[1], Ricardo Tejos[1].
(1) Laboratorio Biotecnología Celular, Recursos Naturales Renovables, Universidad Arturo Prat, Iquique, CL

Reactive oxygen species (ROS) are produced in cells as part of their metabolic process and participate in plant development and responses to the environment, where they play roles in the transduction of hormonal signals and the modulation of cell wall polymer structure, just to mention a few. ROS are important signaling molecules participating in setting the acclimatization response to abiotic stresses in plants, but they are also toxic by-products of stress when they get accumulated inside cells. In the presence of an abiotic stress or such as salt, ROS levels increase in cells, which may cause oxidative damage to the membrane (lipid peroxidation), proteins, DNA and RNA, and can even lead to the oxidative destruction of the cell in a process called oxidative stress. We aim to use *Arabidopsis thaliana* as a model organism to search for metabolites able to ameliorate oxidative stress in plants. For doing so, we isolated several microalgae from different environments of the Tarapacá Region and produced axenic cultures. Microalgae are sources of valuable compounds such as pigments, lipids, carbohydrates, among others, and we hypothesize some of them may have antioxidant capacity hence benefit plants in the presence of abiotic stress. We evaluate the salt-stress tolerance and ROS production in *Arabidopsis thaliana* seedlings. Microalgae metabolite treatments are applied to plants subjected to different concentrations of NaCl to determine the stress-induced ROS production using different methods (TBARS, FOX, DAB staining, NBT staining). We will present the microalgae collection and preliminary results of the bioprospection.

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Structure-based design, synthesis and biological evaluation of novel oxadiazole derivatives as selective 11beta-hydroxysteroid dehydrogenase reductase inhibitors

Gonzalo Recabarren-Gajardo[1], Andrea Vecchiola[2,3], Benjamín Diethlem-Varela[1], Génesis Martínez-Flores[4], Fidel Allende[5], González Pablo M.[1], Fardella Carlos E.[2,3], Lagos Carlos F[4].
(1) Department of Pharmacy, Facultad de Química y de Farmacia, Pontificia Universidad Católica de Chile, Santiago, CL
(2) Department of Endocrinology, School of Medicine, Pontificia Universidad Católica de Chile, Santiago, CL
(3) IMII, Millennium Institute on Immunology and Immunotherapy, Santiago, CL
(4) Chemical Biology & Drug Discovery Lab, Facultad de Medicina y Ciencia, Universidad San Sebastián, Santiago, CL
(5) Department of Clinical Laboratories, School of Medicine, Pontificia Universidad Católica de Chile, Santiago, CL

Overexpression of 11β-HSD1 in key metabolic tissues is related to obesity and metabolic syndrome. In our previous study, we reported the computer-aided identification of adamantyl-triazole derivatives that presented excellent in vitro selective 11β-HSD1 inhibitory potency. To further investigate the structure-activity relationships of these compounds, the triazole ring was transformed to an oxadiazole ring to design a series of adamantly-oxadiazole derivatives. These compounds exhibited in vitro 11β-HSD1 inhibitory potency with IC50 values ranging from 1.1 nM to 145 nM, and do not inhibit the 11β-HSD2 isoenzyme or the 11β-HSD1 oxidase activities at 100 nM. Compounds BD44 and BD40 emerged as candidates for further characterization. In vitro and in silico characterization of ADME/Tox properties were performed to predict the likelihood of the candidate compound to survive successive stages of development. Determination of plasma protein binding was performed for BD40, which displays high plasma protein binding percentages (98,3 ± 1,9%), with a Papp of 1.3x10-6cm s-1, and is not glycoproteinP substrate. These adamantly-oxadiazole based compounds thus represent novel leads for the development of more active derivatives with improved solubility and pharmacokinetic profile targeting intracellular cortisol levels in obesity and metabolic syndrome.

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EFNB2 and TNFSF8 genes are transcriptional targets of Wnt/β-catenin signaling in hematopoietic precursor cells

Lemus Mattias E. [1], Pizarro Hector R.[1], Verdugo Daniela A.[1], Ugarte Giorgia D.[1], Bustos Bernabé I.[2], De Ferrari Giancarlo V.[1].
(1) Instituto de Ciencias Biomédicas, Facultad de Medicina y Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago, CL
(2) Department of Neurology and Center for Genetic Medicine, Feinberg School of Medicine, Northwestern University, Chicago, Illinois, US

Normal hematopoiesis depends on a controlled transcriptional activity of Wnt/β-catenin signaling and this activity is substantially increased in acute myeloid leukemia (AML). To identify target genes that could be involved in the onset/development of AML, here we examined the whole transcriptional program directed by the signaling cascade in KG1 hematopoietic precursor cells. KG1 cells were treated with or without the Wnt/β-catenin agonist CHIR for 4h and 48h and transcription was examined by RNA-Seq analyses, using the Illumina HiSeq2500 with a depth>120 million pair-end reads (2x50 bp). Raw reads were aligned to human genome GRCh38.p12 using HISAT2 and differential expression genes were analyzed by DESeq2. We found that there were 75 genes differentially expressed after 4h of treatment (padj <0.05; 26 up and 49 down, respectively) and that this number increased after 48h to 631 genes (padj <0.05; 296 and 335, up and down). Interestingly, there were 8 genes significantly overexpressed at 4h that kept their expression levels at 48h, including the EFNB2 and TNFSF8 genes, which we further validated by qPCR experiments. Since EFNB2 encodes a protein that promotes angiogenesis and TNFSF8 is a cytokine related with hematologic malignancies, our results support the hypothesis that deregulated Wnt/β-catenin signaling affects key biological processes related with the onset or the development of leukemia.

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Study of a molecule that interferes in Gβγ binding with the cytoplasmic domain of glycine receptor α1

López Andrés David Esteban [1], Guzmán José Leonardo[1].
(1) Laboratorio de Neurobiología Molecular, Facultad de Ciencias biológicas, Universidad de Concepción, Concepción, CL

Ethanol is the most widely used drug of abuse in the world. Its effects go from desinhibition, headaches, nausea, vomiting, even respiratory depression and death. Recently, the glycine receptor (GlyR) has been identified as one of the targets in which this drug acts, enhancing its inhibitory activity. This mechanism involves the interaction of the cytoplasmic domain of GlyR (GlyR-DC) with the βγ dimer of the G protein (Gβγ). Through bioinformatic studies, molecule M554 was selected, which binds to Gβγ at the same site of interaction for GlyR-DC inhibiting the effects of ethanol in vitro and in vivo. In this project it was studied whether this molecule inhibited the interaction between these 2 proteins. For this objetive, a fusion protein of GlyR-DC and Glutathione S-transferase (GlyR-DC-GST) was expressed and purified. Comparative studies of GST pull-down showed that GlyR-DC-GST retained its ability to interact with Gβγ. At the same time GlyR-DC was incubated with cell extracts, and the affinity of GlyR-DC with Gβγ in the absence and in the presence of 200 µM M554 was compared. Densitometric analysis allowed to determine that the interaction between both proteins effectively decreased in the presence of this molecule. Therefore, these results show that this molecule decreases the binding capacity of Gβγ with GlyR-DC, leaving clear that this is the basal mechanism for the inhibition of ethanol effects and supporting the projections that M554 could have a pharmacological potential to treat acute ethanol intoxication.

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Activation of arginase II by ADMA and homocysteine in hypertensive rats induced by hypoxia. A new model of NO synthesis regulation in hypertensive processes?

Vasthi López[1], Fernando Moraga[2], Elena Uribe[3].
(1) Departamento Cs. Biomédica, Facultad de Medicina, Universidad Católica del Norte, Coquimbo, CL
(2) Departamento de Ciencias Biomédicas, Facultad de Medicina, Pontificia Universidad Católica de Chile, Coquimbo, CL
(3) Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, CL

In recent years the increase in blood pressure at high altitude has become an interesting topic among high-altitude researchers. In our animal studies using Wistar rats, we observed the existence of two groups of rats that exhibit differential physiological responses during hypoxic exposure. These were classified as hypoxia-induced hypertensive rats and non-hypertensive rats. A decrease in nitric acid levels was reported in different hypertension models associated with increased concentrations of ADMA and homocysteine, and we recently described an increase in arginase type II expression. ADMA and homocysteine decrease NO bioavailability, however, it is unknown if ADMA and homocysteine have a regulatory effect on arginase activity and therefore regulate another NO synthesis pathway. Therefore, the aim of this study was to determine basal ADMA and homocysteine levels in hypoxia-induced hypertensive rats and evaluate the effect on arginase II activity. RESULT: Hypoxia-induced hypertension rats had lower nitric acid concentrations than non-hypertensive rats, associated to high concentrations of homocysteine and ADMA. Hypoxia-induced hypertensive rats also presented lower dimethylarginine dimethylaminohydrolase-2 and cystathionine β-synthase levels, which could explain the high ADMA and homocysteine levels. Additionally, we observed that both homocysteine and ADMA had a significant effect on arginase II activation in hypertensive rats. CONCLUSION: ADMA and homocysteine have a double regulatory effect on NO synthesis. The former having an inhibitory effect on eNOS and the latter having a secondary activating effect on arginase type II. We propose that arginase II from hypoxia-induced hypertensive rats is activated by AMDA and homocysteine.

FONDECYT 11075096

Doxycycline induces mitochondrial UPR and prevents hyper-proliferation of hypoxic human pulmonary artery smooth muscle cells

López-Crisosto Camila P[1,2], Verdejo Hugo E[1], Sergio Lavandero[2,3], Castro Pablo F[1].
(1) Advanced Center for Chronic Diseases (ACCDiS), División Enfermedades Cardiovasculares, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, CL
(2) Advanced Center for Chronic Diseases (ACCDiS), Facultad Ciencias Químicas y Farmacéuticas & Facultad de Medicina, Universidad de Chile, Santiago, CL
(3) Department of Internal Medicine (Cardiology Division), University of Texas Southwestern Medical Center, Dallas, US

Introduction:
Pulmonary hypertension (PH) is a serious chronic cardiovascular disease with rapid progression and high mortality. The occlusion of pulmonary arteries due to the hyper-proliferation of pulmonary artery smooth muscle cells (PASMCs) is a main feature of PH. This phenotypic change is accompanied by mitochondrial dysfunction and metabolic energy shift. Current treatments for PH have been mainly focused on promoting vasodilation of pulmonary arteries and controlling inflammation. However, no effective therapy is aimed to control PASMC hyper-proliferation. Recent studies proposed that the mitochondrial unfolded protein response (UPR^{mt}) has a hormetic effect on mitochondrial dysfunction. We hypothesized that doxycycline, a common inductor of UPR^{mt}, prevents hypoxia-induced hyper-proliferation in cultured human PASMCs.

Methods:
Human PASMCs were incubated with doxycycline (60 µg/mL) for 4 h and then subjected to hypoxia for 48 h. UPR^{mt} markers were analyzed by RT-qPCR. Cell viability was assessed by flow cytometry. Nuclear ki67, evaluated by immunofluorescence, was used as a marker of cell proliferation.

Results:
Human PASMCs treated with doxycycline showed increased mRNA levels of UPR^{mt} markers (CHOP, C/EBPβ, ClpP, LONP1) with a peak at 48 h of treatment. Cells subjected to hypoxia for 48 h also exhibited a hyper-proliferative phenotype that was prevented by pre-incubation with doxycycline for 4 h.

Conclusions:
Doxycycline induces mitochondrial UPR and prevents the hyper-proliferative phenotype in human PASMCs subjected to hypoxia.

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Golgi phosphoprotein 3 is a non-canonical RAB1A and RAB1B effector

Cavieres Viviana A.[1], Cristóbal Cerda[2], Andrés Rivera-Dictter[1], Rodrigo Castro[1], Charlotte Luchsinger[1], Natacha Santibáñez[1], Burgos Patricia V.[2], **Mardones Gonzalo A.** [1].
(1) Fisiología, Medicina, Universidad Austral De Chile, Valdivia
(2) Centro CEBICEM, Medicina y Ciencia, Universidad San Sebastián, Santiago, CL

Introduction:
GOLPH3 is a trans-Golgi network protein implicated in several aspects of membrane trafficking, and is also considered an oncoprotein. However, it is not well understood how GOLPH3 participates in these mechanisms. Interestingly, GTP promotes the association of GOLPH3 to Golgi membranes and vesicles. Nevertheless, it remains largely unknown whether this response is consequence of the function of GTP-dependent regulatory proteins, such as proteins of the RAB, ARF, or ARL families of small GTPases. Thus, we set to determine whether GOLPH3 is an effector of RAB proteins.

Methodology:
We performed a mini yeast two-hybrid (Y2H) screen between GOLPH3 and several RABs. The interactions found were further characterized by mutational analysis using Y2H assays, GST pulldown, GFP trap, and isothermal titration calorimetry. To investigate the functional role of the interactions, we performed RNAi-mediated knocking-down of RABs in cultured cells, and analyzed the effects on GOLPH3 by subcellular fractionation, immunoblot analysis and fluorescence microscopy, and of GFP-GOLPH3 by live cell imaging.

Results:
The Y2H, GST pulldown and GFP trap assays showed that GOLPH3 binds to RAB1A and RAB1B in a non-canonical fashion. The knocking-down of RAB1A or RAB1B perturbs the subcellular distribution of GOLPH3 and GFP-GOLPH3, which are located in distinct, unidentified, dynamic structures/organelles within the cell. **Conclusions:** GOLPH3 behaves like a non-canonical effector of RAB1A and RAB1B.

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Effect of salt-tolerant rootstocks issued from interspecific cross between cultivated and wild relative halophyte tomato on vegetative growth and physiological parameters in “Old Limachino Tomato” plants under saline stress conditions

Juan Pablo Martinez[1], Farias Karen[1], Salinas Luis[1], Badilla Danitza[2], Rosales Camila[2], Lizana Carolina[2], Sagredo Boris[3], Muriel Quinet[4], Lutts Stanley[5].
(1) INIA-La Cruz, Grupo de Investigación en Fisiología y Biología Molecular Vegetal (GIFBMV), INIA, La Cruz, CL
(2) Instituto de Producción y Sanidad Vegetal, Universidad Austral de Chile, Valdivia, CL
(3) INIA-Rayentue, INIA, Rengo, CL
(4) Earth and Life Institute (ELI/ELIA) , Groupe de Recherche en Physiologie végétale (GRPV) , Université catholique de Louvain, Louvain la Neuve, BE
(5) Earth and Life Institute - Agronomy (ELI-A), Groupe de Recherche en Physiologie végétale (GRPV), Université catholique de Louvain, Louvain-La Neuve, BE

The “Old Limachino Tomato” (OLT) is considered as a salt-sensitive local variety and salinity drastically reduces fruit yield and quality in relation to the deleterious impact of NaCl on mineral nutrition. Grafting procedure has been recommended as a promising strategy to improve salt resistance in this species. The hypothesis of this study is that the salt tolerant rootstocks issued from interspecific cross between cultivated Solanum lycopersicum and wild relative halophyte S. chilense improves the salinity tolerance in OLT grafted plants under saline conditions (NaCl) through higher K+ absorption in relation to Na+ exclusion at the root level. Therefore, the purpose of this study is to investigate the effect of salt tolerant rootstocks issued from interspecific cross between cultivated S. lycopersicum and wild relative halophyte S. chilense on K+ and Na+ contents at the root and shoot levels. Grafted, self grafted and non grafted OLT plants was cultivated at different levels of salinity. Saline stress was imposed seven days after transplant by adding different concentrations of sodium chloride (NaCl) to nutritive solution. The control treatment was set up without NaCl. Saline stress was set up at 80 and 160 mmol L-1 of NaCl in the nutritive solution for 21days. The effect of rootstock and salinity was evaluated on the growth of shoots and the mineral content (Na+ and K+) in roots and leaves under salt stress conditions. Grafted OLT observed higher K+ and lower Na+ contents than other kinds of plant,associated with an increase the salinity tolerance in this vegetable.

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Principles of protein structure, from hypertext to html5 version to visualization in mobile telephone

J Martinez-Oyanedel[1], Felipe Morales[2], Marcos Palma[2].
(1) Departamento de Bioquimica y Biologia Molecular, Facultad de Ciencias Biologicas, Universidad de Concepción
(2) Centro de Formación y Recursos Didácticos, Universidad de Concepción, Concepcion, CL

The current Biology knowledge has been supported by the availability of atomic structures of the molecules that participate and/or interact in the different cellular processes. The ability of students to understand the relationship between structure and function of these macromolecules is the basis for the interpretation of most biological phenomena at the molecular level. The teaching of biological macromolecules has represented a challenge for academics who must face explaining these concepts to undergraduate students: the chemical logic of their composition, how it determines their conformation and how it is influenced by their environment, they must also deliver three-dimensional vision of these molecules with didactic resources in two dimensions. Protein Structure Principles, is a web application that allowed students to review the theoretical contents and also interact directly with the molecules, being able to observe them in 3D, manipulate it and interact with them. The original technological development of this web application is based on a plugin that is not compatible with current viewers (Mozilla, Firefox, Safari, etc.), which has left this application obsolete, affecting around 400 students per semester that cannot use this tool to complement their lecture. This project updates the application to an html5 based system for viewing on mobile and desktop devices. In addition to recovering the application, the project allowed updating the contents and including a self-assessment tool. We will present the application and the evaluation by students, regarding aesthetics, contents and usefulness to understand the protein structure

Proyecto de docencia 2017007

Phycobiliproteins from *Gracilaria chilensis* as sensitizers of DSSC

J Martinez-Oyanedel[1], Paulraj Manidurai[2], Ambbar Aballay[1], Marta Bunster[1], Cristian Agurto[3], Ramon Arrue[3], Ximena Steinberg[1].
(1) Laboratorio de Biofisica Molecular, Departamento de Bioquímica y Biologia Molecular, Facultad de Ciencias Biologicas, Universidad de Concepción, Concepcion, CL
(2) Departamento de Física, Fac. Ciencias Físicas y Matemáticas, Universidad de Concepción, Concepcion, CL
(3) Gibmar, Centro de Biotecnología, Universidad de Concepción, Concepcion, CL

At present there is an urgent need to replace non-renewable energy sources such as fossil fuels with alternative sources of renewable and non-contaminated energy. Solar energy can be directly transformed into electrical energy thanks to the photoelectric effect, through the use of solar or photovoltaic cells. DSSC (dye sensitized solar cells) are one of them. Aquatic organisms such as red algae and cyanobacteria, have a highly efficient protein light capture system called Ficobilisoma. Red algae Phycobilisomas are composed of phycobiliproteins such as Allophycocyanin, Phycocyanin and Phycoerythrin, and binding or linker proteins that help in the assembly and conduction of solar energy. The abundance of red algae in nature, their cultivation potential and the unique spectroscopic characteristics of these phycobiliproteins, make them excellent candidates for semiconductor sensitization in DSSC devices. The objective of this work was evaluated the use of phycobiliproteins obtained from *Gracilaria chilensis*, as sensitizers for DSSC. Phycobiliproteins were extracted from the red algae *G. chilensis*, and purified by chromatographic techniques. The photoelectrode was sensitized by absorbing the phycobiliproteins on the TiO2 semiconductor and the cells were packed with I/I3 as electrolyte. The efficiencies obtained are compared with literature data, showing that the cells have yields similar to those obtained with natural dyes from terrestrial plants. These cells have the particularity of being able to maintain efficiency with low intensity light which makes it possible to use them indoor applications.

Proyecto IDeA ID17I10314

Analysis of gene modulation induced by a high-fat diet in the transcriptome of the POMC and AgRP neurons of the hypothalamus

José Medina Moraga[1,2], Elena Uribe García [2], Estefanía Tarifeño [2], María De Los Ángeles [1].
(1) Departamento Biología Celular, Facultad de Ciencias Biológicas , Universidad de Concepción, Concepción, CL
(2) Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, CL

High-fat diet (HFD) intake produces an imbalance in energy homeostasis, which is maintained through a complex network of signals, where neurons in the arcuate nucleus of the hypothalamus play an important role. Two main neuronal populations are found in this region that control feeding behavior by stimulating appetite (AgRP neurons) or inhibiting appetite (POMC neurons). However, it has been shown that obesity disrupt the proper functioning of this regulatory circuit. Through the use of public scRNA-seq data and bioinformatics analysis, we performed a cell-type characterization of the transcriptomic changes induced in POMC and AgRP neurons of mice fed with a high-fat diet during a week. Differential expression analysis showed that about 65 genes are modulated in POMC neurons, while about 89 genes in AgRP neurons. Through functional enrichment analysis, we identified several biological processes modulated by diet such as *generation of precursor metabolites and energy*, *response to oxidative stress*, *regulation of innate immune response*, *DNA conformation change*. These results indicate that short periods of HFD are sufficient to induce moderate transcriptomic modulation related to metabolic process in both POMC and AgRP neurons. These results together enlighten the biological process affected by obesogenic diets.

VRID-iniciacion from Universidad de Concepcion (No. 218.037.024-1.0)

Interaction between cholesterol and selenium metabolism in salmon macrophages and their effect on susceptibility to infection with *Piscirickettsia salmonis*

Madelaine Mejías[1], Ignacio Chávez[1], Karina Díaz[1], Liliana Montt[1], Javiera Pérez[1], Francisca Vera[1], Dinka Mandakovic[1], Rodrigo Pulgar[1].
(1) Laboratorio de Genómica y Genética de Interacciones Biológicas, Instituto de Nutrición y Tecnología de los Alimentos (INTA), Universidad de Chile, Santiago, CL

The facultative intracellular bacterium *Piscirickettsia salmonis* causes severe losses to the salmon industry in Chile. It has been shown that the abundance of cholesterol is relevant for the infection of intracellular pathogens like *P. salmonis*, which is diminished by the action of statins. On the other hand, selenium has been linked to a better response by the host to intracellular infections by regulating the oxidative state of the fish. In mammals, cholesterol synthesis has a cross-talk with selenoproteins, where the use of statin and selenium induces changes in the expression of selenoproteins and cholesterol-associated genes, respectively. In this study, the cholesterol and selenium genes of Atlantic salmon (*Salmo salar*) were bioinformatically identified and characterized and their transcripts abundances were evaluated in a SHK-1 salmon cell line at different concentrations of selenium and atorvastatin. Also, not cytotoxic nor antibiotic concentrations of sodium selenite and/or atorvastatin were evaluated for SHK-1 cells and *P. salmonis*. Finally, by *in vitro* infections, we observed how selenium and atorvastatin interfered with the resistance of SHK-1 to infection with *P. salmonis*. Forty two genes associated with cholesterol and 36 selenoproteins were identified in *Salmo salar*. Also, we could demonstrate a cross-talk between genes associated with cholesterol and selenoproteins in *Salmo salar*. The concentration of 1 uM of atorvastatin and 1 uM sodium selenite were not cytotoxic nor antibiotic, and generated a decrease in the cytopathic effect of 72.8 % and 76.5 %, respectively. These results could lay the groundwork for new treatments against *P. salmonis* in the salmon production industry.

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Hydroxyproline-rich glycoproteins (HRGPs) affected by overexpression of *PrMADS10* in *Arabidopsis thaliana*

Tamara Ivonne Méndez[1], Raúl Herrera[1].
(1) Instituto Ciencias Biologicas, De Ciencias, Universidad de Talca, Talca, CL

PrMADS10 a transcription factor isolated from Pinus radiata D. Don, is differentially expressed in response to inclination (2 hours after treatment). PrMADS10 gene was overexpressed in Arabidopsis thaliana in order to identify its role in modulating expression of genes associated to cell wall formation. Hydroxyproline-rich glycoproteins (HRGPs) were a superfamily protein identified by microarray experiment. This protein family is related to a diverse aspect like: cell expansion, root growth and development, xylem differentiation, signaling,different stresses and pathogen responses. Are subclasified as: proline-rich (PRP), extensins (EXT) and arabinogalactan proteins (AGPs). Three AGP antibodies were used to determine the localization and quantitation of this protein in radiata pine young seedlings. A total of 23 different proteins were identified. Putative Cis elements were identified in the promoter region for each gene, specifically for response element to MADS-box-NAC-WRKY-AREB-MYC. In up-regulated PRP 5 genes presented cis element response to WRKY and NAC, EIN is less present, and all down regulated genes have NAC and less presence of AREB. All EXT genes presented cis element response to WRKY, NAC, MADS-box, MIC and AREB either both in up and down regulated. The putative sites of DREB and EIN are less representative in this group of genes. Finally, in the case of AGPs group 1 genes no MADS-box Cis element was observed, but the other putative element were present in the promotor region. The presence of this HRGPs modify the proportion of lignin in the cell wall, in this sense, the over-expresion of PrMADS10 could regulate the expression for most of this genes.

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Physiological characterization of 13 winter wheat cultivars released by INIA breading program from 1965 to 2017

Ana-Maria Mendez-Espinoza[1], Miguel Garriga[1], Ivan Matus[2], Dalma Castillo[2], Claudio Jobet[3], Alejandro Del Pozo[1].
(1) Centro de Mejoramiento Genético y Fenómica Vegetal, Facultad de Ciencias Agrarias, Universidad De Talca, Talca, CL
(2) CRI-Quilamapu, Instituto de Investigaciones Agropecuarias, Chillan, CL
(3) CRI-Carillanca, Instituto de Investigaciones Agropecuarias, Temuco, CL

Wheat yields in different regions of the world have increased strongly since the 1960s as a result of genetic improvement and better agronomic practices. With the Green Revolution semi-dwarfing genes were introduced in breeding programs leading to a reduction in plant size and an increase in the partitioning of the above-ground biomass to spikes and grains, associated with increases in harvest index and number of grains per spike and per m2. The present work aimed to evaluate the physiological trait associated with grain yield progress. A set of 13 winter wheat cultivars and advanced lines released in the country between 1965 and 2017 were evaluated at Santa Rosa (36°31' S; 71°54' W), INIA-Quilamapu, in 2018/19. The result showed that the progress in grain yield was associated with higher thousand kernel weight, kernel per spike and harvest index, but not with the number of spike per m2. Modern cultivars had lower non-photochemical fluorescence quenching (NPQ) and specific leaf area, but higher content of chlorophyll and Alpha (the initial slope of the light curve, related to the maximum photosynthetic yield), compared with older ones. In this experiment, no correlation between leaf gas exchange and the year of release was found.

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Functional and transcriptomic characterization of cisplatin-resistant AGS and MKN-28 gastric cancer cell lines

Bárbara Mora-Lagos[1,2], Ismael Riquelme[3], Kurt Buchegger[1], Tamara Viscarra[1], Louise Zanella [1], María Reyes[1], Monserrat Hernández[1], Carmen Ili[1], Priscilla Brebi[1].
(1) Laboratory of Integrative Biology (LIBi), Scientific and Technological Bioresource Nucleus- Center for Excellence in Translational Medicine (BIOREN-CEMT), Universidad de La Frontera, Temuco, CL
(2) Dirección de Investigación. Vicerrectoría de Investigación y Postgrado, Universidad Autónoma de Chile, Temuco, CL
(3) Instituto de Ciencias Biomédicas, Facultad de Ciencias de la Salud, Universidad Autónoma de Chile, Temuco, CL

Introduction:
Gastric cancer (GC) is an important cancer-related cause of death worldwide. The most used chemotherapeutic regimen in GC is based on platinum drugs such as cisplatin (CDDP). However, the main problem of platinum drug treatment is the development of a resistant phenotype associated with a poor prognosis. The aim of this study was to characterize new models of CDDP-resistant GC cell lines (AGS R-CDDP and MKN-28 R-CDDP), in order to understand the molecular mechanisms underlying chemoresistance as well as identify new therapeutic targets for the treatment of GC.

Methodology:
GC cell lines AGS and MKN-28 were obtained through a method based on stepwise increasing drug doses and characterized by cytotoxicity assay (MTT), cell death analysis (Annexin V/Propidium iodine) and relative expression of resistance molecular markers (qPCR). RNA-Sequencing (RNA-seq) was performed on Illumina HiSeq 4000. Sleuth tool was used for differential expression analysis. A fold change >2 and $p<0.05$ were used as a cut-off to choose the differentially expressed genes (DEGs). Gene Ontology (GO) and signaling pathways analysis were analyzed by PANTHER.

Results and conclusions:
Characterization studies have effectively demonstrated that AGS R-CDDP and MKN-28 R-CDDP are reliable CDDP-resistant models. Bioinformatics analyses identified a total of 189 DEGs associated mainly to molecular functions (GO) involved in CDDP-resistance. The most enriched signaling pathway was the inflammation mediated by chemokine and cytokine which could be involved in the development of CDDP resistance in GC. Further studies are necessary to clarify the role of inflammatory processes in GC resistant to CDDP.

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Tracing the evolutive trajectory of allosteric regulation in archaeal enzymes

Sixto M. Herrera[1], Gabriel Vallejos[1], Victor Castro-Fernandez[1], Victoria Guixé[1].
(1) Laboratorio de Bioquímica, Facultad de ciencias , Universidad de Chile, Santiago, CL

In *Euryarchaeota,* the reactions catalyzed by glucokinase (GK) and phosphofructokinase (PFK) use ADP as phosphoryl donor, instead of ATP. In bacteria and eukarya, the PFK reaction has been identified as the main regulator of the glycolytic flux, mainly due to the complex allosteric regulation of this enzyme. However, archaeal PFK enzymes have been described as non-regulated. Interestingly, in *Methanococcales* organisms a bifunctional enzyme able to phosphorylate glucose and fructose-6P has been reported. Moreover, the bifunctional enzyme from *M. maripaludis* was found to be activated by its reaction product, AMP. To asses if this regulatory feature is present in other enzymes form the archaeal ADP-dependent sugar kinases family and to determine if it is an ancestral trait or an evolutionary novelty, we characterized kinetically the effect of AMP on the activity of extant and ancestral enzymes of this family. To determine if this trait is related to bifunctionality, the specific PFK from *P. horikoshii* of the *Thermococcales* order and its ancestor were studied. The results show that AMP activation is present in bifunctional GK/PFK enzymes from the *Methanococcales* order, as well as in the ancestral enzymes of this phylogenetic branch. However, the extant PFK specific enzyme and its ancestor are not activated by AMP, and only the inhibition component of AMP as product is observed. Our results support that the regulatory trait is ancestral and goes along with bifunctionality while lost toward the evolution of specific enzymes, highlighting their relevance in the regulation of GK/PFK bifunctional enzymes

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Extracellular calcium uptake is fundamental for the infection of *Botrytis cinerea Δbcpmr1* mutant strain in tomato

Ignacio Moreno[1], Infante Claudia D.[1], Denisse Carvajal[1], Christian Robles-Kelly[1], Evelyn Silva-Moreno[1,2].
(1) Instituto de Ciencias Biomedicas, Universidad Autónoma de Chile, Santiago, CL
(2) Instituto de Investigaciones Agropecuarias, INIA-La Platina, Santiago, CL

Botrytis cinerea is a main phytosanitary problem to affect Chilean products· Due to the enormous economical relevance, it›s crucial to understand the molecular mechanisms involved in *B. cinerea* infection to develop new control strategies. In this regard, it has been described that the mutant strain *Δbcpmr1* is less effective to infect plants than the wild type. BcPMR1 is an ortholog of a Ca+2/Mn+2-ATPase pump localized in the Golgi apparatus of other ascomycetes and has been associated with calcium uptake. This evidence suggests that the calcium uptake could play an important role during infection. Since the spores can't get calcium from the fungus, it is possible that the calcium required for the early steps of the infection could be obtained from an extracellular compartment, the environment or even from the host?

Methodology:
To measure infection capacity of the mutant strain *Δbcpmr1* under calcium deficient and calcium abundant environments, a conidia suspension of *Botrytis cinerea* strains B05.10 and *Δbcpmr1* supplemented with 10 mM EGTA or 10 mM CaCl2 were used. Wounded and intact leaves and fruits of tomato cv. Micro-Tom were infected with 10 µL of the conidia suspensions.

Results:
The conidia suspension of the mutant strain *Δbcpmr1* supplemented with 10 mM CaCl2 was more infective than the control in all scenarios, and the conidia suspension of the mutant strain *Δbcpmr1* with 10 mM EGTA do not develop infection in intact leaves and fruits.

Conclusions:
Extracellular calcium is required for the infection of the mutant strain *Δbcpmr1* in plant tissues.

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Effect of sucralose in hepatic gluconeogenesis and lipogenesis in mice fed with high fat diet

Omara Moya[1], Pamela Pino[1], Francisco Díaz-Castro [1], Pino Francisco Javier[1], Alejandra Espinosa[2], Rodrigo Troncoso[1,3], Roberto Bravo-Sagua [3,4].
(1) Instituto de Nutrición y Tecnología de los Alimentos , LABINAF, Universidad de Chile, Santiago , CL
(2) Departamento de Tecnología Medica, Facultad de Medicina, Universidad de Chile, Medicina, Universidad de Chile, Santiago, CL
(3) Advanced Center for Chronic Diseases (ACCDiS), Universidad de Chile., CL
(4) Instituto de Nutrición y Tecnología de los Alimentos , OMEGA, Universidad de Chile, Santiago, CL

Introduction:
Obesity is an epidemic disease and a major risk factor for chronic diseases, such diabetes, cardiovascular diseases, cancer and non-alcoholic fatty liver disease. One of the alternative measures to reduce the caloric intake in individuals is the sugar replacement for non-caloric sweeteners. Sucralose is a non-caloric sweetener that have been used to replace sugar in drink, baking and cooking, however there is controversy if sucralose is harmful or safe.

Objective:
Determine the effect of sucralose in hepatic gluconeogenesis and lipogenesis in mice fed with high fat diet.

Materials & Methods:
Mice C57BL/6 wild type were fed by 8 weeks with a Low-Fat Diet (LFD, 10% fat) or High Fat Diet (HFD, 60% fat) and drink water or sucralose (0,1 mg/ml) *ad libitum.* Bodyweight, food intake, maximum aerobic speed, glucose tolerance test (GTT) and pyruvate tolerance test (PTT) were assessments. Liver marker of gluconeogenesis (PCK1, and G6Pase) and lipogenesis (SRBP1, ACC and PAS) were assessment by western blot.

Results:
Sucralose did not affect the body weight, food intake, physical performance and adipose tissue weight. The liver weight was reduced in animals exposed to sucralose. In the animals fed with high-fat diet, sucralose improve the GTT and PTT test. In addition, sucralose reduce the protein levels of PCK1, G6Pase, SRBP1, AC y FAS in the liver of high-fat fed animals.

Conclusion:
These results suggest that sucralose improve the systemic glucose homeostasis in animals fed with high fat diet through a decrease in the hepatic gluconeogenesis and lipogenesis.

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Pairwise probabilistic framework to infer functional gene networks and identify key genes in response to perturbations

Moyano Tomás C [1,2,3], Vidal Elena A[2,4], Eleodoro Riveras[1,2,3], Antoine De Daruvar[5], Gutiérrez Rodrigo A[1,2,3].
(1) Genética Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica De Chile, Santiago, CL
(2) Millennium Institute for Integrative Biology, CL
(3) FONDAP Center for Genome Regulation, CL
(4) Center for Genomics and Bioinformatics, Faculty of Sciences, Universidad Mayor, Santiago, CL
(5) UMR 1332 Biologie du Fruit et Pathologie, INRA, Université de Bordeaux, Bordeaux, CL

It is second nature nowadays to use changes in mRNA levels to identify relevant genes in response to a perturbation or a developmental transition. However, many key genes for an organism's response are not regulated at the mRNA level. These genes are currently hidden to transcriptome-based approaches and require more sophisticated or elaborate experimental strategies to identify them. Here we sought to address the problem of finding functionally relevant genes for a condition "A" using transcriptome data, regardless of whether they change mRNA levels under different experimental conditions to evaluate "A". To identify these genes, we first determined transcriptome states and boundaries using large expression databases and a novel entropy-based framework in two popular model organisms *Arabidopsis thaliana* and *Saccharomyces cerevisiae*. We uncovered inherent restrictions in gene expression at the genome-wide level that reveal novel functional relationships for genes that are not obtained by widely used methods such as correlation networks or mutual information. Moreover, our approach allowed us to identify novel genes in response to perturbations, some of which are and some of which are not regulated in response to the perturbation. Our conceptual framework to analyze transcriptome data complement existing methods and provides new insights into gene regulatory networks that cannot be attained with other methods. This approach can be easily applicable to any organism with large transcriptome databases.

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Palmitate regulates Ire1α in hypertrophied rat cardiomyocytes

Felipe Muñoz[1], Valentina Parra[1], Sergio Lavandero[1,2].
(1) Department of Biochemistry and Molecular Biology, Faculty of Chemical and Pharmaceutical Sciences & Faculty of Medicine University of Chile, Advanced Center for Chronic Diseases (ACCDiS), Santiago
(2) Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, US

Introduction:
Palmitate has been extensively used *in vitro* to mimic the effects of obesity, one of the key factors in the development of heart failure with preserved ejection fraction (HFpEF). Our previous work showed that palmitate treatment for 24 h induces insulin resistance and increases ANP (hypertrophy marker) expression in cultured cardiomyocytes, as well as induces a decrease in cell viability. The ER sensor Ire1α has shown to be a key factor in the physiopathology of HFpEF. In this work we investigate the effect of palmitate on the Ire1α pathway in cultured rat cardiomyocytes in order to establish a link between Ire1α and the metabolic effects of palmitate in the heart.

Methods and Results:
Cultured neonatal rat ventricular myocytes (NRVM) were treated for 24 h with or without DMEM/M199 containing BSA-buffered sodium palmitate 12,5 nM (free fatty acid concentration). An MTT assay and PI incorporation by flow cytometry were used to evaluate reductive homeostasis and cell viability, respectively. Ire1α activation (p-Ire1α/total-Ire1α), insulin resistance (pAKT/total-AKT) and ANP protein level increase (ANP/Tubulin) were determined by Western blot. The results showed that palmitate-treated cardiomyocytes presented insulin resistance, increased levels of ANP, an increased p-Ire1α/total-Ire1α ratio, a decrease in basal Ire1α levels and a less reductive capacity, but without changes in cell viability.

Conclusions:
Palmitate activates Ire1α and decreases its basal levels, together with inducing cultured cardiomyocyte hypertrophy and insulin resistance.

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Crystal structure of the ternary complex of an ancestral ADP-PFK/GK kinase with fructose-6-phosphate and scanning mutagenesis of its active site

Muñoz Sebastián M [1], Nicolás Fuentes-Ugarte[1], Felipe González-Ordenes[1], Pablo Cea[1], Pablo Maturana[1], Victor Castro-Fernández[1], Victoria Guixé[1].
(1) Laboratorio de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Chile, Santiago, CL

Some archaea of the phylum *Euryarchaeota* presents a modified version of the Embden-Meyerhof pathway, where glucose and fructose-6-phosphate are phosphorylated using ADP instead of ATP. These ADP-dependent sugar kinases can be classified as specific for glucose (ADP-GK), for fructose-6-phosphate (ADP-PFK) or bifunctional (ADP-PFK/GK). Although there are several crystal structures for these enzymes, none of them has been determined with fructose-6-P at the active site. The most related work is one where the structure of the ADP-PFK from *Pyrococcus horikoshii* (PDB-3DRW) is reported, which was employed to perform a docking model with fructose-6-phosphate. Among the residues that would contribute to stabilize fructose-6-phosphate at the active site, are Arg191, which is proposed to interact with the phosphate group of fructose-6-phosphate and Arg185 that would interact with the C1 hydroxyl of fructose-6-phosphate. Our group recently described an ancestral ADP-dependent kinase from the order *Methanosarcinales,* able to catalyze the phosphorylation of glucose and fructose-6-phosphate. In this work, we report the crystal structure of this ancestral ADP-PFK/GK determined at 3.12 Å resolution, in ternary complex with fructose-6-phosphate and ADPβS. In this structure, Lys179 interacts with the phosphate group of fructose-6-phosphate instead of Arg191 and Arg185 interacts with the β-phosphate of ADP instead of the C1 hydroxyl of the sugar substrate. To determine the structural determinants for the sugar specificity we perform scanning mutagenesis of the active site residues. These results, along with the crystallographic structure, allow us to determine the critical residues involved in the sugar-binding site of the ADP-dependent kinase family.

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Role of HDAC6 and STAT3 in the expression of PD-L1 receptor in colorectal cancer cells

Camila Navarrete[1,2], Jose Luis Reyes[2], Pia Vidal[2], Matias Hepp[2].
(1) Universidad de Concepción, CL
(2) Laboratorio de Investigacion en Ciencias Biomedicas, Facultad de Medicina, Universidad Católica de La Santísima Concepción, Concepcion, CL

Nowadays colorectal cancer (CRC) is one of the neoplasms with the highest incidence, develops gradually over a long period of time due to accumulation of genetic and epigenetic alterations, generating deregulations, as mechanism of evasion of the immune system. That trigger in tumor cells the expression of some co-stimulatory molecules of immune system, as Programed Death Ligand 1 (PD-L1). It has been reported that the expression of PD-L1 is modulated by STAT3, which is activated by HDAC6. Actually, PD-L1 expression is negatively modulated by HDAC6 inhibitors in other cancers, however, it's not reported in CRC. In this study, we use CRC cells lines (HCT-116 & HT29), to correlate the association between HDAC6 and the activation of STAT3 with the PD-L1 expression. For which, we will affect the activity of HDAC6 using two specific inhibitors (NexturastatA & TubastatinA). Then we evaluated the cytotoxicity, the expression of PD-L1 and the presence of specific post translational modifications on STAT3. After the treatment, we observe that specific inhibitors decrease the cell viability at different concentration in both cells line, but always less than panHDAC inhibitors. Also, we observe that both CRC cells normally express highly mRNA level of PD-L1, which is reduce after the treatment with HDAC6inh, and STAT3 expression is not affected. Finally, we observe the decrease of P-STAT3 after treatment. All these data suggest that HDAC6 affect the activity of STAT3, and the specific inhibition of HDAC6 could negatively modulate the expression of PD-L1, product of the loss of functionality of STAT3.

Ang-(1-9) prevents PDGF-BB induced dedifferentiation in VSMCs

Ignacio Norambuena-Soto[1], Nicole Cancino-Arenas[1], Fernanda Sanhueza-Olivares[1], Paulina Villar-Fincheira[1], Tomás Hernandez-Díaz[1], Mario Chiong[1].
(1) ACCDiS, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile

Introduction:
In atherosclerosis, vascular smooth muscle cells (VSMC) dedifferentiates triggered by platelet-derived growth factor (PDGF), among other hormones. Dedifferentiated VSMC are characterized by increased proliferation, migration and secretion of extracellular matrix. The renin-angiotensin system (RAS) is one of the main regulator of cardiovascular physiology. Recently, a parallel arm of RAS has being described, with antagonistic effects to those described for Ang II. One of these components is angiotensin-(1-9) (Ang(19)). This peptide prevents vascular remodeling in hypertensive rats and this effect is mediated by the activation of the AT2 receptor (AT2R). But it remains unknown whether these protective effects of vascular remodeling are due to a direct effect of Ang-(1-9) on VSMC.

Methods:
Studies were performed in a rat aortic VSMC cell line A7r5. Cells were stimulated with PDGF-BB 20 ng/mL and Ang-(1-9) 1 uM. AT2R was pharmacologically blocked by PD123319. VSMC dedifferentiation was evaluated by measuring the expression of contractile proteins α-SMA and SM22. Cell proliferation was measured by cell cycle determination and migration was evaluated by transwell migration assay.

Results:
PDGF-BB induced at 24 h of treatment an increase in VSMC migration, proliferation and a decrease in contractile protein levels. In contrast, pretreatment of 3 h with Ang-(1-9), abolished all PDGF-BB-induced effects. When PD123319 was used, the prevention effects of Ang-(1-9) were completely blocked.

Conclusion:
Ang-(1-9) prevents the dedifferentiating effects of PDGF-BB on VSMC.

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Characterization of a Novel Phosphatase Activity of Beta-amyloid (1-42) in the Amyloid State

Esteban Nova[1], Octavio Monasterio[2], Rodrigo Díaz[1].
(1) Departamento Biología, Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, CL
(2) Departamento Biología, Facultad de Ciencias, Universidad de Chile, Santiago, CL

The accumulation of beta-amyloid (1-42) (Ab) peptides into amyloid plaques is one of the pathological hallmarks of Alzheimer´s Disease (AD). These plaques are known to contain certain divalent metals such as copper (Cu^{2+}) and zinc (Zn^{2+}). The specific role these metals play in the pathology is a matter of hot debate. In vitro studies show binding of these ions to the peptide through coordination binding with histidine and aspartate residues located in the N-terminal. The recent discovery that rationally designed small peptides can self-assembly into catalytically active amyloids when bound to different divalent metals raises the question whether or not the amyloid state of Ab can also exhibit some catalytic reactivity. In this work we show that pure Ab peptide can self-assemble into amyloids that can hydrolyse p-nitrophenyl-phosphate (pNPP) into p-nitrophenol and phosphate, suggesting the emergence of a potential phosphatase activity. Ab was recombinantly produced in *E. coli* as a pure peptide without modifications using a previously reported purification method that allows for production of high quantities of peptide in a soluble form. The observed activity is strictly associated to the amyloid state of Ab and follows a classical enzymatic-like behaviour characterized by substrate saturation and linear dependence on peptide concentration. Addition of micromolar concentrations of Zn^{2+} caused inhibition of the activity. A detailed characterization of the activity is presented.

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Expression, purification and characterization of a halophilic ADP-dependent kinase from *Nanohaloarchaea*, a new class of archaea

Amparo Núñez[1], Victor Castro[1], Victoria Guixé[1].
(1) Laboratorio de Bioquimica y Biología Molecular, Facultad de Ciencias, Universidad de Chile, Santiago

One of the strategies employed by halophilic organisms is to accumulate inorganic ions inside the cell, which imposes structural modifications of its proteins. In archaea, the halophilic homologs proteins from the class *Halobacteria* and the order *Methanosarcinales,* display very different structural modifications to adapt to salt. Recently, a new group of halophilic nanosized archaea, *Nanohaloarchaea* was reported. These organisms are uncultivable and to date, there are no studies of their proteins or regarding which strategy they employ to adapt to high salt concentrations. We express, purified and characterized an ADP-dependent sugar kinase from halophilic Nanohaloarquea archaeon SW_4_43_9, isolated from the Atacama Desert. The protein presents the conserved sequence motifs associated with the ADP-dependent family. Regarding its conserved sequence motifs, the enzyme was identified as bifunctional, with glucokinase and phosphofructokinase (PFK). The enzyme was cloned and express in the halophilic archaea *Haloferax volcanii* and only the PFK activity was significantly measurable. We determined the kinetic parameters for the phosphofructokinase activity, and its structure by homology modeling. The PFK activity present a dependence with increasing salt concentrations, being the highest activity attained at 2,5M KCl. The enzyme presents hyperbolic kinetics with both substrates, fructose-6P and MgADP, with Km values in the millimolar range, unlike most ADP-dependent kinases reported, however, displays the same structure of other ADP-dependent kinases of the family. This work provides significant insights into the protein characteristics from this new class of archaea and their adaptation strategy to halophilic environments

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Biochemical characterisation of two putative aldose 6-phosphate reductases, AtA6PR1 and AtA6PR2 from *Arabidopsis thaliana*

Karina Olivos-Hernández[1], Michael Handford[1], Juan Carlos Cáceres[2], Ricardo Cabrera[2].
(1) Departamento de Biología, Centro de Biología Molecular Vegetal, Ciencias, Universidad de Chile, Santiago, CL
(2) Departamento de Biología, Laboratorio de Bioquímica y Biología Molecular, Ciencias, Universidad De Chile, Santiago, CL

Plants of the Rosaceae and Plantaginaceae families produce sorbitol during photosynthesis in source organs. This sugar alcohol is then phloem-translocated to sink organs. The enzyme that synthesises sorbitol is aldose 6-phosphate reductase (NADPH) (A6PR), which reduces glucose-6-P to sorbitol-6-P and is the critical regulatory step in the pathway. Sorbitol, which acts as a compatible solute in abiotic stress conditions and facilitates boron mobilisation, is converted to fructose by sorbitol dehydrogenase (SDH) in sink organs. Curiously, A6PR- and SDH-like enzyme activities have been found in families that synthesise and transport sucrose, which is the case of Arabidopsis (Brassicaceae). Two proteins with >65% amino acid identity with known plant A6PRs have been bioinformatically identified and called AtA6PR1 and AtA6PR2. Both proteins have the molecular characteristics of aldo-keto reductase-like proteins, such as three highly-conserved sites: site 1, corresponding to a N-terminal region, site 2, active site region, which contains conserved His, Asp and Tyr, and site 3, the conserved sequence IPKS. Both proteins are cytosolically-localised (GFP-fusion protein), and the expression of both genes is ubiquitous, but they are differentially-expressed under abiotic stress (cold and saline) conditions. Biochemical characterisation of these two proteins was initiated with the expression of codon-optimised His-tagged AtA6PR1 and AtA6PR2 in the heterologous system of *E. coli* BL21 (DE3) plysS. After optimising the induction conditions, the recombinant proteins were purified, and it was possible to determine the activity of AtA6PR1 and AtA6PR2. Work is being performed to evaluate the kinetic parameters and substrate specificity of both enzymes.

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Effects of chronic intermittent hypobaric hypoxia on rat feeding behavior. Role of leptin and HIF-2α at hypothalamic level

Stefany Ordenes[1], Patricia Siques[1], Julio Brito[1], Silvia Arribas[2], María Del Carmen Gonzalez[2], López De Pablo Angel Luis [2], María Rosario López[2].
(1) Institute of Health Studies, Universidad Arturo Prat, Iquique, CL
(2) Department of Physiology, Faculty of Medicine, Universidad Autónoma de Madrid, Madrid , ES

Background:
Pro-opiomelanocortin (POMC) exerts an important anorexigenic effects and has been found to be stimulated during acute hypoxic condition by a Leptin-dependent (*p*-STAT3)- and -independent (HIF-2α) pathways. In chronic intermittent hypobaric hypoxia (CIH) anorexia and weight loss is observed, but is not well known if leptin and HIF-2α pathways would play a role. The objective was to evaluate the effects of AH and CIH (acute period of 12 hours and chronic period 30 days) on: 1) rat feeding behaviour, 2) leptin secretion and 3) associated hypothalamic feeding regulatory pathways.

Methods:
Wistar rats (n=32) were randomly separated into 3 types of exposure: acute hypoxia (4600 m, 12 hours, AH); chronic intermittent hypobaric hypoxia (4600 m altitude, 2 days at hypoxia/ 2 days at normoxia, CIH), and, normoxia (sea level, NX). Body weight and food intake (g) were obtained every 4 days along the experimental period. Plasma Leptin was measured at day 0, 12 hours and day 30. HIF-2α, *p*-STAT3 and POMC proteins levels were measured at 12 hours and day 30 in the hypothalamus.

Results:
Both AH and CIH exposures induced a reduction in weight and food intake. Leptin, *p*-STAT3, HIF-2α- and POMC expression are increased at acute phase (12 hours) while this increase was not observed after chronic exposure (at day 30). **Conclusion:** Acute exposure to hypoxia induces reduction of food intake and weight loss which could be explained by POMC activation through Leptin-*p*-STAT3 and HIF-2α; however, these pathways seem to be blunted after CIH.

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Phytochrome-rapidly regulated 1 (PAR1) participates in carotenoid biosynthesis and photomorphogenic development in carrot

Angélica Ortega[1], Daniela Arias[1], Claudia Stange[1].
(1) Centro de biología molecular vegetal, Facultad de Ciencias , Universidad de Chile, Santiago, CL

Carotenoids are isoprenoid pigments that provide color to flowers and vegetables. They participate in photosynthesis and protect molecules from photooxidative damage caused by excess of solar radiation.Carotenoid synthesis is induced by light during development (photomorphogenesis) of plants. *Daucus carota*, synthesizes and accumulates large amounts of carotenoids in its storage root grown in dark and contrary to other plants, light inhibits the synthesis of these pigments and development of the storage root. To understand the molecular processes that regulate the synthesis of carotenoids in the carrot root, we generate a de novo transcriptome between the root grown in light (R/L) and darkness (R/O). Surprisingly, among the genes overexpressed in R/O we found some that are regulated by light, such as PAR1, a transcriptional cofactor that promotes photomorphogenesis in *A. thaliana* and produces dwarf plants when overexpressed. AtPAR1 interacts with PIF family transcription factors, allowing the expression of phytoene synthase (*PSY*) gene, promoting carotenoid synthesis. We determined that DcPAR1 binds to AtPIF7 and knock-down Dc*PAR1* carrots present a decrease in carotenoids in the root. Here we show the functional characterization of Dc*PAR1* through expressionin *A. thaliana.* Transgenic T3 seedlings with a high level of expression of *DcPAR1* presenta dwarf phenotype with a reduced hypocotyl length and shorter flowering. They also showed an increment in *AtPAR1* and *AtPSY* expression as well as higher level of carotenoids. Our results indicate that *DcPAR1* participates in the synthesis of carotenoids and photomorphogenic development.

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Phycocyanobilin parametrization for functional and conformational studies by molecular dynamics

Diego Ortiz Lopez[2], Ignacio Lizana Bastías[1], Aleksei Delgado[1], Jose Martinez-Oyanedel[2].
(1) Laboratorio de Quimica Teorica Computacional, Facultad de Ciencias Quimicas, Universidad de Concepción, Concepción, CL
(2) Laboratorio de Biofisica Molecular, Departamento de Bioquímica y Biologia Molecular, Facultad de Ciencias Biologicas, Universidad de Concepción, Concepcion, CL

Prosthetic tetrapyrrolic groups are underpinning part of biological processes in vegetal life because they are involved in energy transfer, photosensitivity, and cellular signaling mechanisms. One of these compounds, Phycocyanobilin (PCB), shows different functionalities closely related to its conformation and protein environment. In red algae and cyanobacteria, PCB is bound to two proteins, Allophycocyanin and Phycocyanin, where its function is related to capture and transfer of luminous energy from phycobilisome antenna complex to photosynthesis reaction center. In plants and green algae, PCB is bounded to phytochromes where presents a different conformational behavior and protonation state in comparison with phycobiliproteins. To contribute to the functional and conformational analysis of PCB in red algae and cyanobacteria, we develop a new parameters set compatible with force field CHARMM22. The strategy was to optimize an available parameter set created and validated for PCB in phytochrome context, through a consistent CHARMM parametrization protocol. These new parameters were used in a molecular dynamics study of the Allophycocyanin-Linker Core complex of *Gracilaria chilensis*. To study conformations, flexibility and pyrrolic rings coplanarity, we made a conformational clustering analysis with k-medoids algorithm applied on keys dihedral angles, was necessary written a python code based on MSMBuilder and MDTraj libraries. These results will enable to make new studies of phycobiliproteins containing PCB, through molecular dynamics techniques.

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TNF-α preconditioning induces cardiomyocyte VCAM-1 expression and protection against ischemia/reperfusion injury

Jafet Ortiz-Quintero[1], Troncoso Mayarling F.[1], Valeria Garrido-Moreno[1], Camila López-Crisosto[1], Ramón Corbalán[2], Sergio Lavandero[1,3], Lorena García[1].
(1) Advanced Center for Chronic Diseases (ACCDiS), Faculty of Chemical and Pharmaceutical Sciences & Faculty of Medicine, University of Chile, Santiago, CL
(2) Faculty of Medicine, Pontifical Catholic University of Chile, Santiago, CL
(3) Department of Internal Medicine, Southwestern Medical Center, University of Texas, Dallas, Texas, US

Introduction:
Tumor necrosis factor-α (TNF-α) induces cardiomyocyte death by apoptosis. However this cytokine, at low concentrations, also stimulates cardiac protection against ischemia/reperfusion (I/R) injury. Moreover, TNF-α induces the expression of vascular cell adhesion protein-1 (VCAM-1). Our previous results showed that VCAM-1 is associated with cardiomyocyte survival in a simulated ischemia model. The present work aims to evaluate whether TNF-α preconditioning stimulates cardiomyocyte VCAM-1 expression to protect against I/R.

Methodology:
Cultured neonatal rat cardiomyocytes were treated with TNF-α (0 to 500 ng/mL) and cell viability was assessed by MTT assay and trypan blue exclusion. Protein and mRNA levels of VCAM-1 were measured by Western blot and RT-qPCR, respectively. Then, cardiomyocytes were pre-treated with 10 ng/mL TNF-α for 3-24 h and incubated in ischemic conditions for 6 h. Later, ischemic medium was replaced by DMEM/M199 containing 10% FBS and cardiomyocytes were exposed to normoxia conditions for 16 h. Cell viability was assessed at the end of reperfusion. Student t-test or ANOVA with Tukey´s post-test was performed as appropriate, p value < 0.05 was considered statistically significant.

Results:
The results showed that TNF-α treatment (concentrations < 100 ng/mL for 24 h) did not stimulate cardiomyocyte death. TNF-α induced VCAM-1 expression (protein and mRNA) in cardiomyocytes. TNF-α preconditioning for 6 h protected cardiomyocytes from simulated I/R.

Conclusions:
These results showed TNF-α preconditioning protects cardiomyocytes against I/R injury and this effect could be mediated by VCAM-1.

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Implementation and evaluation of a spectrophotometric protocol in microplates to measure the critical micellar concentration of lipopolysaccharides

Maricarmen Osses[1], Felipe Rojas[1], Belén Navarro[1], Simón Correa[1], Macarena Alarcón[1], Hegaly Mendoza[1], Daniel Aguayo[1].
(1) Center for Bioinformatics & Integrative Biology, Ciencias de la Vida, Universidad Andrés Bello, Santiago, CL

The outer membrane is the first physical barrier of Gram negative bacteria. It is composed of 80% lipopolysaccharides (LPS), an amphiphilic molecule structurally composed of Lipid A, a central oligosaccharide and the O-antigen. Modifications in the LPS promote changes in the membrane permeability and fluidity, indicating changes in the supramolecular structure. The membranes stability modulated by several physicochemical factors, one of these is the surface tension which is directly related to the formation of micellar aggregates. A spectrophotometric protocol in microplates was here developed,implemented and evaluated to determine Critical Micellar Concentration. This protocol is based on the changes in absorbance associated with the curvature of the meniscus formed in the liquid-air interface. Analyzed through the differences Absorbance Radial Profiles (PRA) among each microplate well and its relation with LPS concentration. The data were adjusted to a non-linear regression model of sigmoid type, characterizing each midpoint and slope curve, reflecting the meniscus curvature. Furthermore, the results were compared with changes on the surface tension, calculated by Young-Laplace equation from LPS containing pendant-drops methods. The results reported a change in the meniscus slope curvature that increases until reaching the CMC, being a new indicator for the determination of the CMC from the absorbance values. The protocol implementation developed in this work is easy to implement in a research center, it is fast and reduces the costs of CMC analysis. Finally, the methodology implemented can be used to evaluate the differences in the CMC between different LPS chemotypes or other amphiphilic molecules.

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Transcriptome characterization of intestines of adult zebrafish males of high and low growth after fed with a soybean meal-based diet

Sebastián Pacheco[1,2], Valenzuela Luis V.[2,1], Nicole Díaz[2,1], Natalia Lam[1], Cristian Araneda[1], Pilar Ulloa[1,3].
(1) Laboratorio de Genética y Biotecnología en Acuicultura , Facultad de Ciencias Agronómicas , Universidad de Chile, Santiago, CL
(2) Escuela de Bioquímica , Facultad de Medicina y Ciencias, Universidad San Sebastián, Santiago, CL
(3) Facultad de Medicina Veterinaria y Agronomía, Universidad de Las Américas, Santiago, CL

The limited availability of fishmeal in aquaculture has forced the industry to find alternative protein sources, and soybean meal (SBM) is commonly-used substitute. However, in cultured fish and zebrafish, SBM induces intestinal inflammation, interfering with health and growth. Contrasting impacts have been reported on growth in the same fish species exposed to the same SBM % in feed. This suggests that there is a genetic variability underling the more tolerant fish to SBM diet, favoring growth. However, the relationship of the intestinal inflammation with the growth fish has not been studied. With the selection of SBM-tolerant fish it could be identify the genes that confer intestinal tolerance to a SBM diet. Our initial approach was to feed a population of 19 zebrafish family with 50SBM diet from juvenile to adult (2 months). Males from low growth and high growth were selected (50 mg vs 180mg) to carry out RNA-seq assays. Intestine tissue was used to evaluate the transcriptomic differences. Statistical analysis showed 81 differentially expressed genes (DEGs) between individuals of high and low growth. The main enriched KEGG pathway was “Progesterone-mediated oocyte maturation”, and concordantly, using Gene Ontology database, the most over-represented Biological Process were those related to “Reproductive Process” and “Gamete Generation”. These could be explaining in part, the observed growth differences indicating that in adult males, the plant protein affects the sexual maturation more than inflammatory processes. However, considering that growth is a polygenic character, these DEGs may be potential biomarkers to study an herbivore diet-tolerant fish strain.

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Oxidative and inflammatory markers in endurance athletes

Paredes Aaron J. [1], Silva-Agüero Juan F. [1], Nicole Bruneau[1], Lorena García[1], Luigi Gabrielli[2], Mario Chiong[1].
(1) ACCDiS. Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad De Chile, Santiago, CL
(2) ACCDiS. División de Enfermedades Cardiovasculares, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, CL

Historically exercise has been considered a good practice to maintain cardiovascular health and to prevent cardiovascular diseases. A high intensity endurance training leads to multiple adaptive changes which 5% of the cases are pathological, known as Phidippides cardiomyopathy. However, most biomarkers and techniques to assess cardiac damage do not work properly in trained athletes. In this work two groups of male athletes with low (50 - 99 Km/week) and high (> 99 Km/week) intensity training, from 26 to 50 years old, were blood tested for inflammation and oxidative stress, and assessed by echocardiography previous (basal) and immediately after finishing the marathon of Santiago (post marathon). An increase in post marathon soluble vascular cell adhesion molecule-1 (sVCAM-1) plasma levels compared to control and basal ($p<0.05$) was found. Moreover, this increase was more marked in high intensity trained athletes vs low intensity ($p<0.05$). The soluble interleukin-6 receptor (sIL-6R) showed a mild increase post marathon ($p<0.001$). Higher trained athletes showed a higher change ratio of sIL6-R (pre to post marathon) than lower trained athletes ($p<0.05$). No changes in malondialdehyde (oxidative stress marker) plasma levels were observed between groups. Therefore, higher trained athletes showed an increase in sVCAM-1 and sIL-6R after running the marathon, probably as an adaptive response triggered by a higher training level. Further work will be required to determine whether this inflammation biomarkers could be used to assess pathological adaptation to exercise.

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Structure-function relationships of hemichannels formed by Cx50: How does the IC Pocket affect the ionic flux along the pore?

Claudia Pareja[1], Viviana M. Berthoud[2], Peter J. Minogue[2], Tomas Perez-Acle[3].
(1) Facultad Ciencias de la Vida, Universidad Andrés Bello
(2) Department of Pediatrics, University of Chicago, Chicago IL, US
(3) Computational Biology Lab, Fundación Ciencia & Vida, Ñuñoa, CL

Connexin50 (Cx50) is a gap junction protein expressed in the eye lens that is important for the maintenance of lens transparency. Gap junction channels are made by the coaxial apposition of two hemichannels, each formed by six monomers. To understand the structure-function relationship of Cx50 hemichannel, specifically the molecular basis of key components controlling its flow of ions, we performed all atoms molecular dynamics simulations on wild type Cx50 and on hemichannels formed by Cx50 containing mutations of some of the amino acid residues lining the IC pocket (i.e. R33E, E162R and R33E-E162R). We modeled the ionic currents flowing across them, pore profile and other structural differences, electrostatic potential, and free energy profile to study the passage of an ion through these channels. Compared with wild type Cx50, the single charge reversal mutants affected ion diffusion across the hemichannels and the radius of the pore. Interestingly, the R33E-E162R mutant increased ion diffusion without inducing major changes in the pore radius, but the electrostatic potential profile along the pore on this mutant is different from WT. Last, the free energy profiles are similar between hemichannels wild type Cx50 and the hemichannels formed by Cx50 containing R33E-E162R mutant. The free energy profiles suggest that ion flux could be modulated by alterations of the electrostatic potential on the IC pocket of Cx50 hemichannels. Thus electrostatic potential is a key component for the flux of ions trow this channels.

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Vascular regulation of H_2S synthesizing enzymes by *in vitro* and *in vivo* hypoxia

Paz Adolfo A. [1], Fabián Vega[1], Estefanía Peñaloza[1], Giussani Dino A.[2], Krause Bernardo J.[1].
(1) Neonatology, Pontificia Universidad Católica de Chile, Santiago, CL
(2) Physiology, Development & Neuroscience, University of Cambridge, Cambridge, GB

Presenting Author Email Address: adolfopazf@gmail.com

Introduction:
Hypoxia regulates the expression of genes involved in vascular function. Recently, generation of cysteine-derived H_2S has been suggested as a vasoactive agent. However, its potential regulation by hypoxia and cysteine has not been reported. We studied *in vivo* the effect of N-acetylcysteine (NAC) and hypoxia on the expression of H_2S (CSE, CBS) and NO (eNOS) synthesizing enzymes in chicken embryos, as well as their *in vitro* regulation in human artery endothelium (HUAEC).

Methods:
Fertilized eggs were incubated in normoxic (21% O_2) or hypoxic (14% O_2) conditions. From day 13, embryos were treated with NAC (33 $\mu g*kg^{-1}$) or vehicle (saline). On day 19, embryos were euthanized, and the aorta was removed. Human umbilical artery endothelial cells (HUAEC) were cultured in hypoxia (1% O_2) for 48 hours. CSE, CBS and eNOS transcripts levels determined by qPCR. Potential CSE regulation by HIF was predicted using MatInspector.

Results:
Hypoxic embryos exhibited an increased eNOS expression, which was reduced by the NAC treatment. Both, NAC-treated and hypoxia-incubated embryos showed an increased CSE expression. CBS expression was not altered by any condition. Hypoxia-exposed HUAEC exhibited an increased CSE expression, whilst MatInspector suggested the presence of three binding sites for HIF in both, human and chicken CSE gene promoters.

Conclusions:
CSE expression is regulated by its substrate and by hypoxia *in vitro* and *in vivo*, potentially via HIF.

Oxidative status and protein kinases in right ventricular hypertrophy in rats exposed to chronic intermittent hypobaric hypoxia

Eduardo Pena[1,2], Patricia Siques[1,2], Julio Brito[1,2], Silvia Arribas[3], Rainer Böeger[2,4], Juliane Hannemann[2,4], Maria Del Carmen González[3], María Del Rosario López[5], Ángel Luis López De Pablo[3].
(1) Institute of Health Studies, Universidad Arturo Prat, IQUIQUE, CL
(2) Institute DECIPHER, German-Chilean Institute for Research on Pulmonary Hypoxia and its Health Sequelae, Universidad Arturo Prat , Iquique, CL
(3) Department of Physiology, Medicine, Universidad Autónoma de Madrid, Madrid, ES
(4) German-Chilean Institute for Research on Pulmonary Hypoxia and its Health Sequelae, University Medical Center Hamburg-Eppendorf, Hamburg, DE
(5) Departamento de Medicina Preventiva y Salud Pública , Medicine, Universidad Autónoma de Madrid, Madrid, ES

Background:
Right ventricular hypertrophy (RVH), is one effect of exposure to chronic hypobaric hypoxia (CHH). However, the molecular mechanisms involved are still unclear, and the effects of a new kind of exposure, long-term chronic intermittent hypobaric hypoxia (CIHH), are unknown. Reactive oxygen species (ROS)-mediated pathways may be of major importance, but little is known about their differential expression during CIHH in the left and right ventricles, respectively.

Methods:
Male Wistar rats were randomly subjected to 3 types of exposure: chronic hypoxia (CHH), intermittent hypoxia (CIHH; 2 days hypoxia and 2 days normoxia), and normoxia (NX), for 30 days. After the exposures, were measured in the both ventricles: hypertrophy; lipid peroxidation; the expression of NADPH oxidase subunits (NOX2 and NOX4), p47phox, LOX-1, HIF-1α, and SOD3; and the activity of redox-sensitive protein kinases (p38MAPK and Akt). **Results:** Under CIHH exposure, rats developed RVH and increases in hematocrit, LOX-1 and NOX2 expression, lipid peroxidation, and p38 MAPK activity. However, HIF-1α expression in the RV was observed in both hypoxic groups, but an increase of stabilization in the LV was only observed in the CIHH group. No changes in SOD3 levels were found.

Conclusion:
Long-term CIHH-induced RVH, depicts an increased NOX2 expression and MDA concentration without changes in SOD3 bioavailability might activate hypertrophic pathways (involving LOX-1, p38 MAPK and HIF-1a pathways). Additionally, the LV and RV exhibited different responses to this type of exposure. These findings provide general information that might prompt new analysis of heart under this new type of hypoxia exposure.

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Increased dietary availability of selenium in rainbow trout (*Oncorhynchus mykiss*) improves its plasma antioxidant capacity and resistance to infection with *Piscirickettsia salmonis*

Javiera Pérez[1], Pablo Salgado[2], Daniela Ortiz[2], Liliana Montt[1], Mejías Madelaine[1], Ignacio Chávez[1], Francisca Vera[1], Dinka Mandakovic[1], Jurij Wacyk[2], Rodrigo Pulgar[1].
(1) Laboratorio de Genómica y Genética de Interacciones Biológicas, Instituto de Nutrición y Tecnología de los Alimentos (INTA), Universidad de Chile
(2) Laboratorio de Nutrición Animal, Facultad de Ciencias Agronómicas, Universidad de Chile, Ciencias Agronómicas, Universidad de Chile, Santiago, CL

The main infectious disease affecting the salmon industry in Chile is the Rickettsial Salmon Septicemia (SRS), a pathology whose etiological agent is the intracellular bacterium *Piscirickettsia salmonis*. This disease causes millionaire losses to the industry, being rainbow trout (*Oncorhynchus mykiss*) the species of salmonids more susceptible. The efforts to combat this disease have been unsuccessful, since SRS has prevailed for more than 20 years in Chile. One strategy to address this pathology is to improve the antioxidant response of the host through the supplementation of diets with selenium, an essential micro-nutrient capable of enhancing the antioxidant response of fish. In the present work we evaluated the effect of three diets supplemented with concentrations of 1, 5 and 10 mg of selenium per Kg of diet on the growth of *Oncorhynchus mykiss* and the antioxidant capacity in the plasma during a period of 60 days. Subsequently, we evaluated the protective effect of plasmas against infection with *Piscirickettsia salmonis*in *ex vivo* trials. The results indicate that the dietary supplement of 5 mg Se/Kg improves the growth of the salmonids, the selenium content and also the antioxidant capacity of their plasmas during the course of the trial. In addition, the plasmas obtained from fish fed with this diet at day 30 and 60, showed a protective effect against the infection of *Piscirickettsia salmonis*, indicating that a dietary supplement of 5 mg Se/Kg could be an attractive alternative to combat the SRS in the industry.

Fondecyt de Iniciación 11161083

Cytokinin-dependent transcriptional regulation of PIN auxin efflux carriers in response to developmental and environmental cues

Martín Pincheira[1], Maria Simásková[2], Eva Benková[3], José O'Brien [1].
(1) Genética Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, CL
(2) Department of Plant Biotechnology and Bioinformatics, Ghent University, Ghent, BE
(3) Institute of Science and Technology Austria, AT

PIN-FORMED (PIN) transporters are key components of the polar transport of the phytohormone auxin. It's polar transport is essential for the proper development of the plant since it drives the auxin accumulation in specific cells and tissues. This accumulation, known as auxin maxima, is then necessary for the physiological outcome of auxin, such as root growth and development of lateral root primordia among others. It's been shown that the expression of different PIN transporters is modulated under cytokinin and stress responses such as salt. In response to cytokinin, this modulation takes place in a regulatory element in the PIN promoter denominated *PIN CYTOKIKIN RESPONSE ELEMENT*(PCRE). Thus, we identify the transcription factor PinR4 that is expected to bind to the PCRE domain and could be a component of the crosstalk between this two phytohormones in root development and stress responses. We studied the root architecture -root length and lateral root density- in two mutant lines for PinR4 in response to cytokinin, auxin and salt stress treatments. Our preliminary results show that the *pinr4* mutants are resistant to cytokinin treatment in terms of lateral root density when compared to wild type. Moreover, under auxin treatment the mutant lines showed a reduced density of lateral roots in comparison to wild type. For the salt treatment the mutant lines showed an increase sensitivity as they have shorter roots and had less density than wild type. This result suggests that PinR4 plays a role in the auxin-cytokinin crosstalk in root development and in salt stress response.

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Sucralose prevents the reduction in mitochondrial respiration induced by high-fat diet in mice

Pamela Pino[1,2], Omara Moya[1,2], Francisco Díaz[1,2], Francisco Pino[3], Alejandra Espinosa[3], Rodrigo Troncoso[1,4], Roberto Bravo-Sagua[2,4].
(1) Laboratorio de Investigación en Nutrición y Actividad Física (LABINAF), Instituto de Nutrición y Tecnología de los Alimentos (INTA), Universidad de Chile, Santiago, CL
(2) Laboratorio de Obesidad y Metabolismo Energético (OMEGA), Instituto de Nutrición y Tecnología de los Alimentos (INTA) , Universidad de Chile, Santiago, CL
(3) Departamento de Tecnología Médica, Facultad de Medicina, Universidad de Chile, Santiago, CL
(4) Advanced Center for Chronic Diseases (ACCDiS), Universidad de Chile, Santiago, CL

Introduction:
Obesity has reached a pandemic status worldwide. Its alarming prevalence has led to an increase in the use of non-caloric sweeteners as sugar replacement, specially sucralose. Although sucralose is considered safe, its effect on liver mitochondrial metabolism remains unknown.

Objective:
To determine the effect of sucralose on hepatic mitochondrial function in high-fat diet (HFD) fed mice.

Subjects & Methods:
3-weeks old male C57BL/6 mice were fed ad libitum for 8 weeks with control diet or HFD, and either with water or water supplemented with sucralose (0,1 mg/mL). Apart from body and liver weight and food/drink intake, we also measured mitochondrial respiration rate (using a Clark electrode) and mitochondrial protein markers (PGC1alpha and mtHSP70 through western blot) in liver.

Results:
Sucralose did not affect food or drink intake or body weight gain during the 8-week treatment. However, it prevented both the decrease in mitochondrial respiration and the increase in liver weight induced by HFD. Moreover, HFD induced an increase in PCG1alpha, which sucralose also prevented.

Conclusions:
These results suggest that HFD induces a decrease in mitochondrial metabolism and an increase in liver weight. Given that PGC1alpha stimulates mitochondrial biogenesis, we hypothesize that is an adaptive response to mitochondrial dysfunction. Sucralose intake rescued mitochondrial metabolism, which correlates with a prevention of liver weight and PGC1alpha increase.

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Visualization Software for the statistical analysis in the formation of secondary DNA structures under conditions of directed evolution: *Cry11Aa* case study

Efrain Pinzon-Reyes[1,2], Francisco Adasme-Carreño[2], Jans Alzate-Morales[2].
(1) Instituto Masira, Grupo Biología Molecular y Biotecnología, Medicina, Universidad de Santander, Bucaramanga, CO
(2) Departamento de bioinformática, Facultad de Ingeniería , Universidad de Talca, Talca, CL

The directed evolution technique, also known as DNA shuffling, has been successfully used to obtain new protein variants with improved characteristics. DNA shuffling makes use of the modified PCR technique to obtain recombined genes, where the primers are replaced by small fragments of the two parental genes to recombine. It is precisely the use of simple strand fragments as a primer, which allows the intrinsic characteristics of the DNA of each parental gene to govern the shuffling process. We believe that in this technique the formation of DNA secondary structures of these strands gives preference to the recombination of some gene regions, which may have evolutionary purposes. We have calculated the frequency and energetics of the formation of DNA secondary structures under DNA shuffling using the UNAFold software, which combines free energy minimization, partition function calculations and stochastic sampling for folding prediction. We have developed a software tool that allows the statistical visualization of the data associated with the formation of DNA secondary structures in experimental evolutionary techniques. The *cry11Aa gene of Bacillus thuringiensis* has been studied using this software. The collected data can be transferred to the *Cry11Aa* protein and will be useful to predict the possible evolutionary preferences between the three structural domains conserved in the *Cry11* family, reported in the literature. This information is a valuable input for further work related to the rational design of new *Cry* variants.

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Challenges in the sampling and refinement of RNA structures using multiscale simulation approaches for structure prediction

Simón Poblete[1,2], Bernhard Thiel[3], Giovanni Bussi[4], Ivo Hofacker[3].
(1) Instituto de CIencias Físicas y Matemáticas, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, CL
(2) Computational Biology Lab, Fundación Ciencia & Vida, Santiago, CL
(3) Institute for Theoretical Chemistry, University of Vienna, Vienna, AT
(4) Statistical Biophysics Group, SISSA, Trieste, IT

The knowledge of the three-dimensional structure of a given RNA molecule is crucial for the understanding of its biological function. Taking in consideration the complexity of its experimental determination, computational modelling emerges as a powerful tool to perform this task. Among them, the simpler, coarse-grained models are particularly interesting, due to their comparative efficiency with respect to all-atom simulations for the exploration of the conformational space.

Some of us have recently introduced the SPlit and conQueR (SPQR) model, a nucleotide-level representation of RNA, having successfully predicted several important motifs and incorporating non-canonical base-pairs, glycosidic torsions and sugar puckers. In addition, Ernwin consists of a coarser, helix-centered description, as another model which allows to sample larger structures (of the order of hundreds of nucleotides) but with a lower resolution, dealing with junctions, stems and tertiary contacts between them. The proposed prediction procedure is, starting from a given sequence, to sample its tertiary structure using the low-resolution Ernwin scheme, identify and refine the lowest-energy structures at the nucleotide-level SPQR scheme and finally, to reconstruct the these structures in an all-atom representation, to present them as candidates for the native structure.

We report here the preliminary results of this multiscale approach applied to three different test structures, between 66 and 159 nucleotides, and show how to efficiently reintroduce atomistic details, remove topological artifacts and clashes introduced by the sampling scheme and how to identify tertiary contacts with our software in a matter of a few seconds or minutes.

candidates for the native structures

Fondecyt Iniciación 11181334

Study of Phytochrome A in carotenoid synthesis and development of the carrot storage root

Rocío Quian[1], Christian Gonzalez [1], Luis Felipe Quiroz[1], Claudia Stange[1].
(1) Centro de Biología Molecular Vegetal, Facultad de Ciencias, Universidad de Chile, Santiago, CL

In plants, carotenoids participate in photosynthesis and protect molecules against photo-oxidative damage. Light promotes carotenoid synthesis through an increment in carotenogenic genes expression given by the activation and translocation to the nucleus of photoreceptors, such as PHYA. In carrot, contrary to most plants, carotenoids are produced in the storage root grown in darkness and light impairs carotenoid synthesis and root development. To understand the genetic regulation in carrot root, we performed a de-novo RNA-seq between root grown in light (R/L) and in darkness (R/O). Surprisingly, genes related to light signaling, such as Dc*PHYA* are overexpressed in R/O. PHYA is activated and stable under Far-red (Fr) light. In *Arabidopsis*, Fr is transmitted to the background and At*PHYA* transcript is up-regulated in the root. To understand the role of Dc*PHYA* in carotenoid synthesis and development of the carrot storage root, we determined the relative transcript levels of Dc*PHYA* in R/L and R/O in orange and white carrot roots at three stages of development. We show that Dc*PHYA* is up-regulated in R/O in all development stages in the orange variety. Dc*PHYA* is also mostly expressed in orange root than in the white one. These results suggest that Dc*PHYA* could be involved in carotenoids synthesis in the orange variety. *In silico* analysis showed that DcPHYA has a 73% of amino acid identity with AtPHYA in their functional domains. To evaluate the role of Dc*PHYA*, we overexpressed, performed knockdown and knockout in carrot. Phenotypic, molecular and biochemical analysis of seedlings will be shown.

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Biochemical Characterization of Two Single Mutant (A+ and Nefza) that Give Rise to a Glucose-6-phosphate dehydrogenase A- Double Mutant

Ramírez Edson Jiovany Nava [1], Yadira Yazmín Cortés Morales[3], Laura Eloísa Morales Luna[2], Víctor Martínez Rosas[3], Leyda Cortés Maldonado[3], Beatríz Hernández Ochoa[4], Paulina Ortíz Ramírez[3], Alejandra Abigail González Valdez[5], Jaime Marcial Quino[6], Saúl Gómez Manzo[3].
(1) Posgrado en Ciencias Biológicas, Medicina , Universidad Nacional Autónoma de México, Mexico City, MX
(2) Posgrado Ciencias Biológicas, Medicina , Universidad Nacional Autónoma de México, Mexico City, MX
(3) Bioquímica Genética, Instituto Nacional de Pediatría, Mexico City, MX
(4) Laboratorio de Inmunquímica, Hospital Infantil de México Federico Gómez, Mexico City, MX
(5) Departamento de Biología Molecular y Biotecnología., Instituto de Investigaciones Biomédicas. Universidad Nacional Autónoma de México, Mexico City, MX
(6) Instituto Nacional de Pediatría- Laboratorio de Bioquímica Genética, Consejo Nacional de Ciencia y Tecnología, Mexico City, MX

Introduction
Glucose-6-phosphate dehydrogenase (G6PD) is a key regulatory enzyme that plays a crucial role in the regulation of cellular energy and redox balance. Mutations in the gene encoding G6PD cause the most common enzymopathy that drives hereditary nonspherocytic hemolytic anemia.

Methodology
To gain insights into the effects of mutations in G6PD enzyme efficiency, we have investigated the biochemical, kinetic, and structural changes of three clinical G6PD variants, the single mutations G6PD A+ (Asn126AspD) and G6PD Nefza (Leu323Pro), and the double mutant G6PD A− (Asn126Asp + Leu323Pro).

Results
The mutants showed lower residual activity (≤50% of WT G6PD) and displayed important kinetic changes. Although all Class III mutants were located in different regions of the three-dimensional structure of the enzyme and were not close to the active site, these mutants had a deleterious effect over catalytic activity and structural stability. The results indicated that the G6PD Nefza mutation was mainly responsible for the functional and structural alterations observed in the double mutant G6PD A−. Our study showed that biochemical and structural changes found in G6PD Nefza and A- variants matched those reported for Class I G6PD variants, suggesting the need to re-classify these mutants which should include clinical and biochemical characteristics of these G6PD variants.

Conclusions
From the solved three-dimensional structure of the human G6PD protein, we defined changes in the interactions of the amino acid that offer a molecular explanation for the effects of these mutations, and provide a molecular explanation for clinical manifestations observed in individuals with G6PD mutations.
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Unifying tanycytes through Cx43: communication and self-renewal

Antonia Recabal[1], Sergio López[1], Roberto Elizondo-Vega[1], Teresa Caprile[1], Christian Steinhäuser[2], María Ángeles García-Robles[1].
(1) Biología Celular, Ciencias Biológicas, Universidad de Concepción
(2) Institute of Cellular Neurosciences, DE

Introduction.
Tanycytes have been postulated as hypothalamic neuronal precursors due to their privileged position in the hypothalamus that allows them to detect mitogenic signals and because they share the characteristics of neuronal precursors located in other neurogenic niches, including formation of coupled networks through connexins. However, the connexins involved in tanycyte coupling remain undescribed. As other neuronal precursors do, it is unkown whether tanycytes use purinergic signaling to regulate and synchronize the cell cycle through calcium waves that occur spontaneously and spread through gap junctions.

Methods.
We evaluated coupling of tanycytes through gap junctions in hypothalamic slices isolated from connexin-specific knock out mouse lines by filling an individual cell with biocytin, a connexin-permeable molecule capable of diffusing to other cells in the presence of a coupled network, during whole-cell patch clamp recording. To test if the purinergic signaling play a role in the tanycytic-self renewal response, primary cultures of tanycytes were exposed to a Cx43 inhibitor and different ATP concentrations, and their proliferation was examined by BrdU incorporation.

Results.
We demonstrated that tanycytes are highly coupled to each other and also give rise to a panglial network specifically through Cx43. Additionally, the proliferative response of tanycyte cultures exposed to mitogenic factors *in vitro* was suppressed by inhibition of Cx43; and it was promoted by extracellular ATP, confirming the participation of purinergic signaling in their self renewal.

Conclusion.
Our results demonstrate the importance of Cx43 in tanycyte homotypic and panglial coupling, which influences the proliferative potential of hypothalamic cells.

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How a second Mg^{2+} ion affects the phosphoryl transfer mechanism and its free energy barrier in a protein kinase

Rodrigo Recabarren[1], Kirill Zinovjev[2], Iñaki Tuñón[3], Jans Alzate-Morales[1].
(1) Centro de Bioinformática, Simulación y Modelado (CBSM), Facultad de Ingeniería, Universidad de Talca, Talca, CL
(2) School of Biochemistry, University of Bristol, Bristol, GB
(3) Departament de Química Física, Universitat de València, Valencia, ES

Mg^{2+} ions are essential for the proper functioning of protein kinases. These metal cofactors bind in the protein's active site restricting the motion of the ATP molecule (when ATP is used as phosphate source), increase ATP/ADP affinity and neutralize the negative charges in the active site. CDK2 (cyclin-dependent kinase 2) was firstly postulated to work efficiently with only one Mg^{2+} ion in its active site, as many other kinases, until a crystal structure with two Mg^{2+} ions was found. This new structure resembled crystal structures of kinases like PKA (protein kinase A) suggesting a common catalytic mechanism with two magnesium ions. In this contribution, the different proposed phosphoryl transfer mechanisms, namely substrate- and base-assisted pathways, in the presence of one and two Mg^{2+} ions were assessed computationally. Free energy barriers were calculated using a QM/MM (quantum mechanics/molecular mechanics) hybrid methodology (adaptive string method) at the DFTB3/Amber level of theory. Our calculations show that, as proposed experimentally, the reaction free energy barrier is slightly higher (about 3 kcal/mol) with one Mg^{2+} ion at the active site and that the base-assisted mechanism is favored. Charge analysis shows that the negative charge on the transferred phosphoryl group is less stabilized in the presence of one Mg^{2+} ion, what generates a higher repulsion with the entering nucleophilic hydroxyl group, increasing the free energy barrier. It is expected that these results may be extrapolated to other structurally related kinases where the influence of a second Mg^{2+} ion in the active site is still under debate.

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Peroxyl radical-oxidation of *Mj*FtsZ generates protein dimerization and functional changes via oxidation of tryptophan, tyrosine and methionine

Juan S Reyes[1], Camilo López-Alarcón[2], Octavio Monasterio[1].
(1) Laboratorio de Biología Estructural y Molecular, Facultad de Ciencias, Universidad de Chile, Santiago, CL
(2) Free radical and proteins oxidation, Faculta de Química y de Farmacia, Pontificia Universidad Católica de Chile, Santiago, CL

The microorganisms have developed the ability to reproduce under extreme environmental conditions. Thermophiles are able to grow at temperatures close to the boiling point of water in niches also exposed to high oxidative stress conditions. In fact, in hot springs, due to the high concentration of salts and metals, reactive oxygen species, as peroxyl radicals, are produced. The polymerization of the protein FtsZ forms a structure called Z-Ring, that is the first step of the cytokinesis. This structure is the scaffold for the rest of the division machinery. The present work deals with the study of the oxidation of thermophilic protein MjFtsZ mediated by peroxyl radicals (generated from thermolysis of AAPH). Exposure of MjFtsZ to a total dose of 240 uM of peroxyl radicals (in 30 min) rapidly induced formation of covalent dimers as evidenced by SDS-PAGE. Together with this, its intrinsic polymeric activity was totally abolished. The monomer consumption by the dimerization was nearly to 50% characterized by chromatography (HPLC-DAD). The inactivation/dimerization of MjFtsZ mediated by peroxyl radicals was related to consumption (assessed through by HPLC-Fluorescence) of susceptible amino acids such as Tyr, Trp and Met. Our results show that peroxyl radicals induce oxidative modifications on specific amino acids of MjFtsZ mediating inter-protein radical-radical or secondary reactions pathways leading to covalent MjFtsZ dimerization.

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Structural study inhibition of α7-nicotinic acetylcholine receptor by antidepressants

Maximiliano Rojas[1,2], Yorley Duarte[1], Jesús García[3], Jonathan Canan[1], González-Nilo Fernando D.[1,2].
(1) Center for Bioinformatics and Integrative Biology, Ciencias de la Vida, Universidad Andrés Bello, Santiago, CL
(2) Instituto Milenio Centro Interdisciplinario de Neurociencia de Valparaíso, Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, CL
(3) Neurobiología Celular y Molecular, Instituto de Neurobiología, Universidad Nacional Autónoma de México, Querétaro, MX

Major depression and nicotine addiction are social devastating health situations. Nicotinic acetylcholine receptors (nAChRs) are pentameric proteins that belong to the superfamily of neuronal nAChRs. The nAChRs are formed of a combination of subunits α, β, γ or ε, which may be assembled as heteromeric proteins, or of only one subunit such as homomeric α7-nAChR. The α7-nAChR is widely distributed in the organism, and it is involved in Alzheimer's disease, epilepsy, depressive disorders, drug addiction, among others. The functioning of nAChRs is modulated by a variety of substances interacting in allosteric sites including cations, local anesthetics, and antidepressants. Is knowledge that several antidepressants inhibit AChRs, but its molecular mechanism is unknown. Therefore, we studied to molecular level the behavior of several antidepressants on α7-nAChR using computational techniques for comparing with the biological activity data obtained by electrophysiology. In this regard, the aim of this work was to study against α7-nAChR the behavior to the molecular level of seven known antidepressants, using computational tools like homology model, molecular docking and molecular dynamics with Schrodinger Suite. The computational results suggest that the affinity of each antidepressant is consistent with experimental results, presenting a complete correlation in their free energy values. The amino acids in the binding site have electronegative characteristics, which increase the probability to form interactions with the cationic antidepressants. Finally, this work is presented as the first structural model of α7-nAChR, which allows us to understand the mechanism of action at the molecular level and their therapeutic relationship actions.

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ADAR1-mediated effects over lncRNAs in triple-negative breast cancer cell lines

Pamela Rojas De Santiago[1,2], Alejandro Blanco[2], Rodrigo Assar[2], Katherine Marcelain[3], Sjöberg Marcela K.[1], Ricardo Armisén[2].
(1) Laboratory of Epigenetic Regulation, Department of Cell and Molecular Biology, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, CL
(2) Center of Excellence in Precision Medicine CEMP, Pfizer Chile, CL
(3) Basic Clinical Oncology, Medicine, Universidad de Chile, Santiago, CL

Adenosine deaminases acting on RNAs 1 (ADAR1) proteins have been described as modulating actors of the expression and function of its targeted coding and non-coding RNAs. Among the latter, long non-coding RNAs (lncRNAs) (>200 bp in length) have emerged as central components in cellular processes both in physiological and pathological conditions. There are few reports in literature that examine ADAR1-lncRNAs interplay. Nevertheless, there is still much to understand on a genome wide scale. In breast cancer, both ADAR1 and lncRNAs have been characterized as key components of cell proliferation, migration and invasion processes, suggesting possible roles in oncogenic transformation and tumor-suppressors pathways. Our work is focused on lncRNAs changes in expression induced by ADAR1 and how this could affect cancer progression. By using RNA-seq, we detected that ADAR1 upregulation in the breast cancer cell line MDA-MB-231 induces the differential expression of 38 antisense, intronic and intergenic lncRNAs genes (p-adj<0.05). The downregulated LINC00944 and APCDD1L-AS1 and the upregulated LINC01003 and H1FX-AS1 lncRNAs were validated through RT-qPCR and further tested in ADAR1 *knockdown* condition. A second triple-negative breast cancer cell line, MDA-MB-436, was used to confirm changes in expression of lncRNAs after ADAR1 up- and downregulation finding the same results. Finally, the LINC00944 knockdown and cell viability, proliferation and migration assays allowed us to determine the contribution of this lncRNA in the ADAR1-mediated phenotype in breast cancer cell lines.

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Chemical composition and biological activity of crude extract obtained from *in vitro* induced callus of *Drimys winteri* (canelo) and *Leptocarpha rivularis* (palito negro)

Julia Rubio[1], Christian Robles-Kelly[1], Guisselle Arias[1], Catalina Álvarez[2], Evelyn Silva-Moreno[3], Andrés F. Olea[4], Héctor Carrasco[4].
(1) Instituto de Ciencias Biomédicas, Universidad Autónoma de Chile, Santiago, CL
(2) Centro de Investigación y Producción de Plantas, CIPP, CL
(3) Instituto de Investigaciones Agropecuarias, INIA - La Platina, CL
(4) Instituto de Ciencias Químicas Aplicadas, Universidad Autónoma de Chile, Santiago, CL

Metabolites presents in native/endemic plant population are relevant due to their interesting biological activity. In this category, two species: *Drimys winteri* or "canelo" (DW) and *Leptocarpha rivularis* or "palito negro" (PN) are prominent because it was possible to isolate from them, compounds with antifungal or cytotoxic activity. In this work, we propose that the phytochemical composition of semipolar crude extract (CEx) obtained from callus induction will be similar to the CEx from an adult plant.

A strategy to extract these compounds and non-intervening in their natural environment was implemented to validate the hypothesis. Therefore, our main goals were to develop *in vitro* culture protocols for calluses induction and maintenance as well as *in vitro* plant propagation of DW and PN. Internodal segments of PN and DW were successfully introduced and propagated in specific culture media. Propagated plantlets and leaves obtained from *in vitro* plants were used for callus induction, the CEx assayed were obtained from these calluses generated under controlled conditions. Their phytochemical composition was determined by TLC and HPLC techniques to assess the CEx biological activity in diverse cellular and fungal model.

Analysis of the composition of CEx from *in vitro* propagated plantlets of PN and induced callus of DW showed the presence of main compounds described for both species. CEx biological activities were challenged over the fungal model *Botrytis cinerea* or in tumoral cells lines. Our first analysis in both biological models showed results that could be used to corroborate our approach.

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Hypoxia and oxidative stress modified HIG2A subcellular localization: HIG2A in the mitochondria retrograde signaling

Celia Salazar[1], Alvaro Elorza [2,3], Lina Ruiz [1].
(1) Instituto de Ciencias Biomédicas , CL
(2) Institute of Biomedical Sciences, CL
(3) Millennium Institute of Immunology and Immunotherapy, CL

Introduction:
Mitochondria produce a diverse number of retrograde signals (from mitochondria to nucleus) to fine-tune cellular metabolism, repair mechanisms or defense responses against different stress factors. Retrograde signals have a direct impact on transcriptional and epigenetics regulation of nuclear genes. HIG-1 hypoxia inducible domain family member 2A protein (HIG2A) has been found in both mitochondria and nucleus. HIG2A mediates the assembly of the mitochondrial respiratory chain supercomplexes, having a role on mitochondrial dynamics and bioenergetics. Furthermore, HIG2A function has been associated with cell proliferation. Our objective is to evaluate the function of HIG2A in the communication between mitochondria and nucleus in conditions of hypoxic and oxidative stress.

Methodology:
The subcellular localization of HIG2A was evaluated in C2C12 and HEK293 cells under stress conditions by immunofluorescence analysis, cellular fractioning, and Western blot.

Results:
HIG2A was found to localize equally between mitochondria and nucleus in basal conditions. However, exposure to oxidative stress or hypoxia caused a shift toward its nuclear localization. *In silico* analysis of HIG2A predicted a nuclear localization signal, DNA-binding residues, and a probable SUMOylating residue. In the nuclei fraction with anti-HIG2A antibody, it was detected an upper band of approximately 10 KDa higher than HIG2A, suggesting a putative post-translational modification of HIG2A.

Conclusion:
HIG2A dynamic distribution between mitochondria and nucleus in response to stress factors, may account for a new mito-nuclear communication system.

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An *ex vivo* culture of human fallopian tube reveals clues about possible embryo-mother crosstalk during the preimplantation window

Pablo Saldivia[1], Mauricio Hernandez [1], Cristian Vargas[2], Guillermo Nourdin[1], Francisco Alvarez[1], Alan Fritz[1], Wendy Garín[2], Stecher Juan F[2], Elard Koch[1].
(1) Division of Proteogenomics, MELISA Institute, San Pedro de la Paz, CL
(2) Clinical Protocol, Division of Epidemiology, MELISA Institute, San Pedro de la Paz, CL

Background:
Embryo-mother molecular crosstalk before implantation is supposed as an essential process for the establishment of pregnancy. There is evidence that viable embryos secrete a specific peptide known as Preimplantation Factor (PIF), which potentially plays a role in the generation of a favorable maternal environment for the trip of the embryo in the fallopian tube until their implantation in the uterus wall. This study aimed to evaluate changes in the proteome of human fallopian tube explants before and after exposure to PIF stimulus.

Methodology:
Fallopian tube explant of a healthy woman was cut in nine sections. This was synchronized and stimulated with culture medium (control) and 100 nM PIF for 16 hours. Total proteins were extracted, digested and tryptic peptides were analyzed by nHPLC coupled to TimsTOFpro platform. Protein identification was performed by *Homo sapiens* Swissprot (560,292 entries) with PEAKS_X. The network and enrichment pathways analysis were performed with g: Profiler, REVIGO, and Cytoscape.

Results:
We identified 2460 proteins, where 188 proteins were differentially expressed in PIF treatment (>1.5-fold change in expression; adjusted p-value < 0.0039, MTC Benjamini-Hochberg). Gene Ontology and Biological pathway analysis of differentially expressed proteins revealed significant enrichment for proteins involved in extracellular matrix organization and modulation of the immune response in PIF treatment. Furthermore, immunomodulation molecule HLA-G was detected by proteomics and western blot only in PIF treatment.

Conclusions:
Our study provides an interesting approach to understand how fallopian tube reacts to early embryo signals. This ex vivo model appears useful to investigate embryo-maternal molecular crosstalk.

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THE SNARE-like superfamily in tomato: identification and transcriptional analysis reveal a possible involvement in salt stress

Josselyn Salinas-Cornejo[1], José Antonio Madrid-Espinoza[1], Simón Ruiz-Lara[1].
(1) Instituto de Ciencias Biológicas, Universidad de Talca, Talca, CL

SNARE plays a major role in membrane fusion for the selection and delivery of their cargoes between the membranous compartments. This becomes very important in conditions of saline stress, since the elimination of ion channels and the different types of plasma membrane transporters, as well as the compartmentalization of toxic ions, require the formation of vesicles. Mass sequencing of plant genomes has revealed a superfamily of proteins called SNARE-like, which seem to be participating in vesicular traffic with functions similar to SNAREs. Cultivated tomato (*Solanum lycopersicum*) is considered one of the most economically important crops and excessive salt accumulation represents a challenge for its cultivation, affecting plant growth and final yield. Nevertheless, a tomato relative *Solanum chilense*, which is tolerant to different abiotic stresses and adapted to extreme environments, provides a tremendous genomic resource to explore stress tolerance. Studies of the participation of SNARE-like in tomato are unknown. Therefore, genes encoding SNARE-like in *S. lycopersicum* plants were characterized. The phylogenetic analysisand the identification of conserved domains were made for its characterization. In addition, the transcript levels by qPCR of a salt-sensitive tomato and a salt-tolerant wild tomato *Solanum chilense* under salt stress were analyzed. The results showed that SNARE-like genes have an early induction under salt-stress conditions. Moreover, the subcellular location of one of this SNARE-like fused to GFP was determined using different organelle-markers fused to mCherry. It is expected that these results serve as a basis for the use of these genes in genetic improvement programs in tomato.
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IGF-1 regulates mitochondrial calcium uniporter (MCU) expression and function in hypertrophied cardiomyocytes

Pablo Sánchez-Aguilera[1], Alexis Díaz-Vegas[2], Ariel Contreras-Ferrat[1], Mario Chiong[1,3], Sergio Lavandero[1,3].
(1) Advanced Center for Chronic Diseases (ACCDiS), Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, CL
(2) Charles Perkins Centre, School of Life and Environmental Sciences, University of Sydney, Sydney, AU
(3) Center for Exercise, Metabolism and Cancer (CEMC), Facultad de Medicina, Universidad de Chile, Santiago, CL

Introduction:
Cardiac hypertrophy is characterized by an increase in cardiac size in response to a wide range of stimuli. Physiological cardiac hypertrophy is a reversible process associated with increased cardiac mitochondrial function. In contrast, pathological cardiac hypertrophy impairs cardiac function and metabolism. Insulin-like growth factor 1 (IGF-1) mediates exercise-induced physiological cardiac hypertrophy. On the other hand, mitochondrial Ca^{2+} uniporter (MCU) supports the mitochondrial calcium (Ca^{2+}_{mt}) uptake and its cardiac content is increased in exercised mice. Interestingly, MCU overexpression stimulates hypertrophy and mitochondrial function in skeletal muscle. However, the effect of IGF-1 on MCU expression/function has not been investigated in the context of cardiomyocyte hypertrophy triggered by IGF-1.

Methodology:
The effect of IGF-1 over MCU expression/function was evaluated by measuring MCU protein levels by Western blot (Wb) and Ca^{2+}_{mt} uptake through fluorescence microscopy in cultured neonatal rat cardiomyocytes treated with IGF-1 (10 nM) for 48 h. Cardiomyocyte hypertrophy was evaluated by fluorescence microscopy measuring the cell area and perimeter.

Results:
Our data show that IGF-1 stimulated AKT phosphorylation and increased cardiomyocyte area and perimeter. Treatment with IGF-1 also increases MCU protein levels (45%) compared to controls as well as MCU-dependent Ca^{2+} uptake assessed by Rhod-2 fluorescence in 2 µM free Ca^{2+} permeabilized cardiomyocytes.

Conclusion:
IGF-1 stimulates cardiomyocyte hypertrophy and increases MCU content and Ca^{2+}_{mt} uptake in cultured rat cardiomyocytes.

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Reduced Angiotensin II Type 2 Receptor expression is associated with gastric cancer progression and receptor activation reduces gastric cancer cell migration

Alejandra Sandoval-Bórquez[1,2], Samuel Martinez[1,2], Pedro Novoa[1,2], Gonzalo Carrasco-Avino [3,4], Alejandro Corvalan[3,5], Sergio Lavandero[1,2,6], Quest Andrew F. G. [1,2].
(1) Advanced Center for Chronic Diseases (ACCDiS), Faculty of Chemical & Pharmaceutical Sciences & Faculty of Medicine, Universidad de Chile, Santiago, CL
(2) Laboratory of Cellular Communication, Center for Studies on Exercise, Metabolism and Cancer (CEMC), Institute of Biomedical Sciences (ICBM), Faculty of Medicine, Universidad de Chile, Santiago, CL
(3) Advanced Center for Chronic Diseases (ACCDiS), Faculty of Medicine, Pontificia Universidad Católica de Chile, Santiago, CL
(4) Department of Pathology, Faculty of Medicine, Hospital Clinico Universidad de Chile, Santiago, CL
(5) Hematology and Oncology, Faculty of Medicine, Pontificia Universidad Catolica de Chile, Santiago, CL
(6) Department of Internal Medicine, Cardiology Division, University of Texas Southwestern Medical Center, Dallas, Texas, CL

Angiotensin II Type 2 Receptor (AT2R), is an effector protein of the renin renin-angiotensin system which may play a role as a tumor suppressor in cancer. AT2R has been implicated in the regulation of several cancer-related cellular processes, including migration and invasion. However, our current understanding of the role of this protein in gastric cancer (GC) remains unclear. The objective of this study was to evaluate the expression of AT2R in GC progression and determine the effect of receptor activation on GC cell migration. We determined the expression of AT2R in precancerous gastric lesions, advanced GC samples and normal tissues next to tumor. Association analysis of the expression of AT2R with clinicopathological features and survival curves were also performed. Finally, the effects of AT2R on cell migration and transmigration were evaluated using human GC cells. We observed that AT2R was downregulated in GC patients, and that decreased expression correlated with disease progression from pre-neoplasic lesions to GC. Moreover, loss of expression of AT2R was associated with depth of tumor invasion and poor overall survival. On the other hand, AT2R activation reduced migration and transendothelial migration of GC cell lines. This study provides insight to the role of AT2R in GC, suggesting a potential role in preventing tumor cell migration and invasion. Further research is required to characterize the role of this protein in GC. We anticipate that the new insights gleaned from studying AT2R function in GC will enable the future design of more effective treatments for this neoplasia.

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L-NAME-induced hypertension is associated with a decrease in aortic contractile proteins

Fernanda Sanhueza-Olivares[1], Valeria Garrido[1], Felipe Muñoz-Cordova[1], Nicole Cancino-Arenas[1], Ignacio Norambuena-Soto[1], Mayarling Troncoso[1], Sergio Lavandero[1], Mario Chiong[1].
(1) ACCDiS. Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad De Chile, Santiago, CL

Introduction:
Hypertension is a chronic pathology affecting 1.3 million people worldwide. Even though actual treatments effectively reduce blood pressure, it still remains as one of the most prevalent risk factors for cardiovascular diseases and a major cause of premature death. Administration of N(ω)-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase (NOS) inhibitor, is a widely used model to induce hypertension.

Aim:
The aim of this study was to evaluate the effect of different concentrations of L-NAME on contractile proteins levels in the aorta in an *in vivo* model of L-NAME induced hypertension.

Methods:
16 week-old C57BL/6N mice were treated with different concentrations of L-NAME (0.25, 0.5 and 1 g/L in drinking water) for 5 weeks. Blood pressure was determined using a non-invasive blood pressure System (NIBP). Aortas were isolated and protein lysates were prepared by mechanical and chemical homogenization of the tissue. Proteins were quantified using the Bradford method and smooth muscle protein 22 (SM22), calponin, alpha smooth muscle actin (αSMA) and ß-tubulin levels were evaluated by Western blot.

Results: After 5 weeks, only L-NAME 1 g/L treated mice developed hypertension. A reduction in calponin and αSMA protein levels at 0,5 g/L and 1 g/L of L-NAME and a reduction in SM22 protein levels at 1 g/L of L-NAME were observed in mice aorta. **Conclusion**: Here we show that vascular alterations occur even before the development of detectable hypertension in mice. Understanding the mechanism involved in the genesis and progression of hypertension is critical for the future development of new treatments for vascular pathologies.

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Direct visualization of structural changes in membrane-integrated GluA2 AMPA receptors by fast-scan AFM imaging

Santander E A [1], Edwardson J M[1].
(1) Department of Pharmacology, University of Cambridge, Cambridge, GB

Ionotropic glutamate receptors are ligand-gated ion channels that mediate the vast majority of excitatory transmission in the central nervous system. AMPA receptors (AMPARs) are mainly postsynaptic tetramers that mediate the effects of the neurotransmitter glutamate. We purified GluA2 AMPARs and reconstituted them in lipid bilayers for fast-scan atomic force microscopy (AFM) imaging. Both AMPAR splice variants, flip and flop, were visualized as two distinct globular structures, corresponding to the 'dimer-of-dimers' arrangement of the amino-terminal domains (ATDs). We found that the ATDs of the flop isoform are more mobile than those of the corresponding flip isoform, indicating differences in the intrinsic flexibility of the resting states of the two isoforms in unstimulated conditions. Surprisingly, this behaviour was interchangeable through the switching of a single amino acid, residue 775, which is serine in flip and asparagine in flop. The mobility of the flip (but not flop) ATDs increased when the receptor was activated by glutamate (10 mM). We suggest that fast-scan AFM imaging represents an excellent approach to study the dynamics of membrane-integrated receptors under near-physiological conditions.

Analysis of cultural materials of biological origin: Genetic characterization of bark cloth textiles

Barbara Peña-Ahumada[1], Mónica Saldarriaga-Córdoba[2], Elizabeth Matisoo-Smith[4], Mauricio Moraga[3], **Daniela Seelenfreund[1]**, Andrea Seelenfreund[5].
(1) Departamento de Bioquímica y Biología Molecular, Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, CL
(2) Cirenys, Universidad Bernardo O´higgins, Santiago, CL
(3) ICBM, Medicina, Universidad de Chile, Santiago, CL
(4) Anatomy, Medicine, University of Otago, Dunedin, NZ
(5) Antropología, Ciencias Sociales, Universidad Academia de Humanismo Cristiano, Santiago, CL

The scope of our research is to reconstruct the settlement history of Oceania through the genetic study of species closely associated to humans. Artifacts of biological origin stored in museum collections are open treasure chests. The genetic study of museum textiles from Pacific islands made of plant fibers can reveal some of these hidden histories. Several species have been used as a source of fiber for textiles. The most valuable textiles are made of the bark of paper mulberry (*Broussonetia papyrifera*), a tree introduced into the Pacific in prehistoric times (5000-1500 BP). In this study, twelve Pacific bark cloth textiles from museum collections were analyzed. The aim of this study was to identify the species used for its manufacture and to characterize the genetic diversity of paper mulberry textiles. We analyzed DNA obtained with nuclear molecular markers. The species used as fiber source was shown to be paper mulberry in seven of the twelve textiles. These seven samples were further characterized with microsatellites and obtained alleles were compared with those previously found in contemporary plants and herbarium leaves. We detected new alleles and specific genetic signatures, indicating greater genetic diversity in the past, compared to contemporary plants and herbarium samples of the same species. These signatures suggest connections between plants of the native region (Asia), Near Oceania and Remote Oceania. These results show that these cultural biomaterials can be characterized using genetic tools, and applied to the reconstruction of the human settlement history of the Pacific.

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CB1R regulates autophagy in skeletal muscle

Carlos Sepulveda[1], Juan Rodríguez[1], Matías Monsalves[1], Camila Donoso[1], Daniela Cota[2], Miguel Llanos[3], Rodrigo Troncoso[1,4].
(1) Laboratorio de Investigación en Nutrición y Actividad Física, Instituto de Nutrición y Tecnología de los Alimentos, Universidad de Chile, Santiago, CL
(2) INSERM U1215, Neurocentre Magendie, Universite de Bordeaux, Bordeaux, FR
(3) Laboratorio de Hormonas y Regulación Metabólica, Instituto de Nutrición y Tecnología de los Alimentos, Universidad de Chile, Santiago, CL
(4) Advanced Center for Chronic Diseases (ACCDiS), Universidad de Chile, Santiago, CL

Introduction:
CB1R is the classical receptor in the endocannabinoid system. It's regulates food intake and metabolism in several tissue including liver, adipose and skeletal muscle. In muscle, CB1R activation reduce glucose uptake and increase lipogenesis. Besides, CB1R activation modulates AMPK, mTOR and MAPK/ERK signaling pathways. Autophagy is an evolutionarily conserved cellular degradation process that targets cytoplasmic materials including cytosol, macromolecules and unwanted organelles. mTOR and AMPK are the main regulators of autophagy, but it's not known if CB1R can regulates autophagy in skeletal muscle.

Objective:
To study the role of CB1R in the regulation of autophagy in skeletal muscle.

Materials and methods:
Two experiments were performed. First, male C57BL/6 *Cnr1+/+* and *Cnr1-/-* were grouped in WT (n=8) and KO (n=7), respectively. Tibial anterior was extracted for analyses of mRNA and protein levels of CB1R and autophagy. Second, male C57BL/6 *Cnr1+/+* and *Cnr1-/-* were grouped in WT-PBS (n=5), WT-CQ (n=5), KO-PBS (n=6) and KO-CQ (n=8). 4 h before sacrifice, 100 mg/kg of body weight of chloroquine was injected to assessment autophagic flux. Likewise, tibial anterior was extracted for analyses of mRNA and protein levels of autophagy.

Results: In the first experiment, no differences on basal autophagy in mRNA and protein levels, neither in level phosphorylation of AMPK, mTOR and ERK 1/2. However, in the second experiment there is an increase in autophagy flux in KO-CQ compare with WT ($p<0.05$).

Conclusion:
Our results suggest that CB1R regulates autophagy in skeletal muscle.

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Influence of the DAMPs methanol and oligogalacturonides over plant-aphid interaction

Christian Silva[1,2], Francisca Blanco-Herrera[2], Ariel Orellana[2].
(1) Centro de Biotecnologia Vegetal, Ciencias Biologicas, Centro de biotecnología Vegetal UNAB, santiago, CL
(2) Centro de Biotecnologia Vegetal, Ciencias Biologicas, Universidad Andrés Bello, Santiago, CL

Besides the intrinsic structural function of the cell wall polymer homogalacturonan (HG), this pectic domain also possesses a key role in plant defense. Infections with pathogens harboring pectin modifying enzymes cause the de-methylation of HG by an increase in pectin methylesterases (PMEs) activity, producing methanol and unmethylated HG chains. Once unmethylated, HG is depolymerized by the action of polygalacturonases (PGs) and/or pectate lyases (PLs), releasing HG oligomers called oligogalacturonides (OGs). Both methanol and OGs are damage-associated molecular patterns (DAMPs) which trigger different defense responses including callose deposits formation, reactive oxygen species (ROS) synthesis, and up-regulation of a wide set of defense genes. Methanol and OGs-mediated mechanism have been characterized for different pathosystems, however, its influence over plant-aphid interaction remain unclear. We previously found that early aphid infestation induces a rise in PME activity and the methanol emissions, concomitant with an increase in PL activity, thus suggesting the participation of a defense response mediated by these DAMPs upon aphid attack in Arabidopsis. Therefore, the present work aims to determinate the influence of methanol and OGs over plant aphid-interaction. To achieve this, we evaluated the colonization performance of aphids in terms of settling preference, population growth, fecundity, and feeding behavior in Arabidopsis plants treated with OGs and/or methanol.

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Glycolysis inhibition by 2-deoxy-D-glucose reduces migration in cancer cells

Layla Simón[1,2], Ricardo Huilcamán[1,2], Lorena Oróstica[1,2], Alejandra Sandoval[1,2], Andrew Quest[1,2].
(1) Laboratorio de Comunicaciones Celulares, Facultad de Medicina, Universidad de Chile, Santiago, CL
(2) Advanced Center for Chronic Diseases (ACCDis), Faculty of Chemical and Pharmaceutical Sciences and Faculty of Medicine, Universidad de Chile, Santiago, CL

Metastasis is the leading cause of cancer-related deaths, making the development of novel, more effective therapies a must to alleviate patient suffering. Metabolic switching is a hallmark of cancer cells that facilitates metastasis. Work from our laboratory has shown that Caveolin-1 (CAV1), a membrane-bound scaffolding protein, promotes migration, invasion and metastasis of cancer cells by activating a novel Rab5-Rac1 signaling pathway. More recent evidence indicates that CAV1 also induces glycolysis and reduces mitochondrial respiration. Therefore, we sought to determine if restriction of glycolysis would lead to reduction of CAV1-enhanced migration/invasion/metastasis, and reversion of metabolic switching. Here we evaluated, as a first step, the effects of 2-deoxy-D-glucose (2-DG, a glycolysis inhibitor) in metastatic cancer cell lines. Non-cytotoxic concentrations of 2-DG inhibited in a dose-dependent manner migration of MDA-MB-231 breast cancer and B16F10 melanoma cells. In addition, 2-DG reduced endogenous CAV1 protein levels in MDA-MB-231 cells. Thus, 2-DG effectively inhibits glycolysis, migration and CAV1-associated metabolic switching. Collectively, these findings highlight the importance of CAV1 in the metabolic reprogramming during metastasis, and point towards possible therapies to prevent metastatic disease by modulating metabolism and CAV1 expression.

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Development of *Vitis vinifera* REN1RUN1 plants resistant to powdery mildew (*Erysiphe necator*)

Viviana Sosa[1], Patricio Arce-Johnson[1].
(1) Molecular biology, Biology, Pontificia Universidad Católica de Chile

Grape is the most important fruit crop worldwide. Each year 75,8 millions of tons are produced and 47% of them are used in wine production, which converts it in its main purpose. Chile is the fourth global wine exporter. The most destructive illness in grape is powdery mildew, that is caused by the *Erysiphe necator* fungus. Currently, the main control of powdery mildew is carried out by chemicals fungicides, which has as consequence high expenses, negative effects in animal and human health and adverse environmental impact. At this moment, there are not grape cultivars with powdery mildew resistant and favorable agronomic features. However, several powdery mildew genes have been described, within them the REN1 locus and RUN1 gen. REN1 has been associated with a decrease in secondary hypha development, ROS production, and reduction in conidiospores. RUN1 is related with programmed cell death and the ROS generation. In table grape, it has been described that the combined action of REN1 and RUN1 generate a stronger resistant to *E. necator* attack than each gen by separate. So, the main goal of this project is to generate *Vitis vinifera* wine cultivars plants resistant to powdery mildew. For this purpose, we made crosses between sensitive and resistant grapevine plant generating a progeny of RUN1REN1 segregating varieties using the backcrossing method. The characterization of powdery mildew resistant plants was through molecular markers and differential expressions of genes related to resistance to pathogens. Also, we characterize to histological level the plants response to powdery mildew attack. Advances in obtaining resistant grapevines plants will be shown.
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Domain swapping and DNA binding properties of FoxP1 by insertion of evolutionarily-conserved residues in the wing 1 region

Stephanie Tamarìn[1], Pablo Villalobos[1], César Ramírez-Sarmiento[2], Exequiel Medina[1], Jorge Babul[1].
(1) Departamento de Biologia, Facultad de Ciencias, Universidad de Chile
(2) Pontificia Universidad Católica de Chile, CL

Forkhead box P (FoxP) proteins (FoxP1– 4) are members of the human Fox transcription factors family, involved in cell development, immunity and tissue homeostasis; they are characterized for adopting quaternary structures via domain swapping (DS), in which each monomer breaks native contacts to exchange with its partner, generating an intertwined structure. Although it has been reported that the hinge region is crucial in this association, there are other elements that have evolved to modulate this process. One region that has not been deeply studied and share both sequence and length diversity between the family members is wing 1, where two highly-conserved consecutive amino acid residues are absent in the FoxP subfamily (glycine 68-proline 69), suggesting their role in the emergence and/or modulation of the association properties in FoxP members. To get insights about these residues, a single glycine and a double proline-glycine insertions were generated to analyze their impact in both DNA– and DS binding processes. Kinetic dissociation studies showed that the single insertion decreases the affinity between monomers, whereas the double insertion increases both kinetic pathways with no changes under equilibrium conditions compared to the wild-type protein. Moreover, anisotropy changes, using a fluorescent-labelled DNA showed that the single insertion highly increases the protein-DNA affinity, whereas the double insertion showed no significant differences compared to the wild-type protein. These results highlight the evolutionary changes in wing 1 related with the specialization of protein-protein association properties and the DNA-affinity in FoxP proteins.

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Tripanocidal activity of chilean seaweeds

Pablo Barraza[1], **César Torres[2]**, Ivania Avalos[1], Javiera Castillo[1], Luis Araya[1], Viviana Heredia[1], Mauricio Alfaro[1], Bessy Gutierrez[1], Luis Tapia[1], Jorge Borquéz[1], Jorge González[1].
(1) Tecnología Médica, Ciencias de la Salud, Universidad de Antofagasta, Antofagasta, CL
(2) Biomedico, Ciencias de la Salud, Universidad de Antofagasta, Antofagasta, CL

Trypanosoma cruzi infect 7-8 million people worldwide and in their chemotherapy, only two drugs are active mainly in the acute phase, also presenting multiple side effects. The tripanocidal effect of algae has been reported, however the activity of Chilean algae has not been evaluated. The objective of this work was: To evaluate the trypanocide activity of seaweed extracts from northern Chile. To do this, hexane, ethyl acetate and methanol extracts of *Gracilaria chilensis*, *Ulva lactuca*, *Lessonia trabeculata*, *Chondrus canaliculatus* and *Chondracanthus chamissoi* were obtained. The tripanocidal activity was evaluated with epimastigotes that express b galactosidase making serial dilutions of the extracts and incubating for 48 h at 37 °C. Then, the enzyme substrate, the red galactosidase fenol-D galactopyranoside was added and the absorbances at 570 nm were determined. The study of the LD50 showed that the extracts with the greatest effect against T. cruzi were those from *C. canaliculatus* showing the highest activity (LD50 2.07 mg/mL), followed by the ethyl acetate extract of *C. chamissoi* (LD50 3.55 mg/mL). On the other hand, *G. chilensis* Hexane: 34.6 mg/mL and *U. lactuca*: Hexane 77.8 mg/mL were the less active. Cytotoxicity against HeLa cells showed that all LD50s were at 170 mg/mL or higher, except the *G. chilensis* hexane extract which was 56.2 mg/mL. It is concluded that Chilean seaweeds extracts have trypanocidal activity highlighting the effect of *C. canaliculatus* extracts.

Network for Extreme Enviromentals Research Proyect (NEXER PROYECT ANT11756, Universidad de Antofagasta , CHILE)

Induction of protective immune response against *Trypanosoma cruzi* infection by immunization with recombinant protein phosphatase 2A (TcPP2A)

Torres César Gianni[1], De Pablos Luis Miguel[2], Bessy Gutierrez[3], Antonio Osuna[2], **Jorge González[3]**.
(1) Biomédico, Ciencias de la Salud, Universidad de Antofagasta, Antofagasta, CL
(2) Instituto de Biotecnología, Universidad de Granada, Granada, ES
(3) Tecnología Médica, Ciencias de la Salud, Universidad de Antofagasta, Antofagasta, CL

Trypanosoma cruzi the causative agent of Chagas disease, infects between 6 to 8 million people worldwide and more than 100 species of mammals in endemic areas and the Americas. Apart from treating rural housing with insecticides, there are no adequate infection control strategies. Treatment is performed only with two drugs licensed during the last century, which have strong side effects, moderate effect during the acute phase of infection, but discreet activity in chronic forms. Although vaccination with prophylactic or therapeutic finers could be a powerful control strategy, there are no vaccines licensed for human or animal use and despite of several different studies, there is no vaccine against *T. cruzi*. Recombinant TcPP2A was used to immunize groups of 5 mice. In all cases, each mouse received three 10 mg immunizations of rTcPP2A in association with Titter Max, with 10-day intervals. After the last immunization, the antibody titers were determined, and the immunized mice were challenged by inoculation of 105 C8C3 clone tripomastigotes. A control group of 5 animals was inoculated with PBS. In all cases the infection course was monitored by studying the parasitemia curve. Observing the curve and analyzing the data obtained, we could see a significant percentage of protection, achieving over 70% of it. Inputting the data in a comparative statistic, we could also observe a significant difference between the control and immunized groups. Based on these results we propose that TcPP2Ar is a new candidate for immunoprophylaxis of *T. cruzi* infection.

Internalization mechanism of folate-modified PAMAM dendrimers is mediated by more than one endocytosis pathway

Josefa Torres[1], Pilar Vásquez[1], Felipe Vidal[1], Leonardo Guzmán[1].
(1) Fisiología, Ciencias Biológicas, Universidad de Concepción, Concepción, CL

Nowadays, central nervous system (CNS) diseases affect 1.5 billion people worldwide and there is a continuous development of new therapies. However, in many cases efficiency of therapies is low because of biological barriers and deficient biodistribution of drugs. New advances in the nanomedicine have allowed the creation of nanotransporter systems. Among them, polyamidoamine (PAMAM) dendrimers have demonstrated a great potential in drug delivery to CNS. PAMAM dendrimers are polymeric structures composed by an ethylenediamine core that branches creating layers, called generations, which end in primary amines protonated at physiological pH and can be modified with other terminal groups, such as folate. Considering the current difficulty of delivering drugs to the CNS, we examined the internalization mechanism of folate-conjugated PAMAM dendrimers mediated by folate receptor α (FRα), a membrane protein overexpressed in choroid plexus that once it binds to folate is internalized by the caveolae endocytosis pathway, and is postulated as a target tissue for drug delivery to CNS. In this study, we selected the HeLa cell line for internalization experiments, based on confocal and western-blot results. One unmodified (G4) and two folate-modified (PFO25 and PFO50) fourth generation PAMAM dendrimers were used. Confocal images showed that PFO50 was not able to entry HeLa cells, unlike PFO25 and G4, which were visualized after one hour incubation. Quantification of Mander's coefficients indicated only a slight increase of colocalization of PFO25 with FRα than unmodified G4, which suggests that the internalization pathway of folate-modified dendrimers is possibly mediated by more than one endocytosis mechanism.
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Identification of mTOR as a putative target of CK2 in colorectal cancer cells

César Trigo-Hidalgo[1], Julio Tapia[1].
(1) Oncología Básico Clínica, Medicina, Universidad de Chile, Santiago, CL

Introduction.
The mammalian target of rapamycine (mTOR) is the core protein kinase in two complexes (mTORC1 and mTORC2) at PI3K/AKT/mTOR pathway. This pathway coordinates inputs from several signaling pathways whose function is the regulation of a variety of cellular processes, such as protein synthesis, cell cycle, autophagy and survival. The protein kinase CK2 has more than 300 substrates described in literature. CK2 is highly expressed and active in several cancers and is currently being considered a target for cancer therapy. In addition, specific inhibition of CK2 with silmitasertib leads to a decreased activity of mTORC1 in colorectal cancer cells. However, whether mTOR is a target of CK2 is unknown.

Methodology.
An *in silico* primary analysis of mTOR was used to identify putative phosphorylation sites for CK2. Once residues were identified, pull-down assay using extracts from HEK-293T non-tumor embryonic kidney and DLD-1 colorectal cancer cells ectopically expressing recombinant mTOR were performed. Both mTOR-CK2 and mTOR-DEPTOR interactions were evaluated after treatment with silmitasertib.

Results.
Some residues were found in mTOR which are located into the FAT domain and involved in its interaction with DEPTOR, an integrant of both mTORC1 and mTORC2 complexes, which functions as a negative regulator of the catalytic subunit. Inhibition of CK2 with silmitasertib mainly affected the protein-protein interactions in DLD-1 colorectal cancer but not HEK-293T embryonic cells.

Conclusion.
Protein kinasemTOR is a putative target for CK2 in colorectal cancer cells. Phosphorylation by CK2 may regulate mTOR activity by controlling its interaction with DEPTOR.

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Cardiac VCAM-1 expression is increased both in *in vitro* and *in vivo* inflammation models

Mayarling Francisca Troncoso[1], Jafet Ortiz-Quintero[1], Pablo Esteban Morales[1], Ramón Corbalán[2], Sergio Lavandero[3,1].
(1) Advanced Center for Chronic Diseases (ACCDiS), Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, CL
(2) División de Enfermedades Cardiovasculares, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, CL
(3) Deparment of Internal Medicine, University of Texas South Western Medical Center, Dallas, Texas, US

Introduction:
The prevalence of obesity, insulin resistance and diabetes mellitus has increased during the last years. Chronic and low grade inflammation is present in all these diseases and also associated with the development of diabetic cardiomyopathy and heart failure. However, the molecular mechanisms associated with their development remain still poor understood. VCAM-1 is an endothelial transmembrane sialoglycoprotein involved in the transmigration of inflammatory cells from the blood to the tissues. Its role in cardiac inflammation and link with obesity is not fully understood. To this end, our aim was to investigate whether VCAM-1 expression is increased in cultured cardiomyocytes treated with TNF-α and also in an experimental model of obesity.

Methods & results:
Cultured neonatal rat ventricular myocytes (NRVM) were treated with TNF-α for 24 h and in the last 30 min exposed to insulin in order to establish an in vitro inflammatory model. The expression of phosho-AKT, AKT and VCAM-1 were determined by RT-qPCR and/or Western blot. The results showed that VCAM-1 protein levels were increased (1.5 times) in NRVM treated with TNF-α but without changes in the activation of Akt. Cardiac VCAM-1 protein levels were also increased (0.5 times) in C57BL/6 male mice fed with high-fat diet for three months.

Conclusion:
These results suggest that cardiac VCAM-1 expression is increased both in vitro and in vivo inflammation models.

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Adenosine and β-catenin crosstalk: Regulation of stemness in proneural and mesenchymal Glioblastoma Stem-like Cells

Daniel Uribe Brange[1], Ángel Ayuso[2], Claudia Quezada[1].
(1) Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral De Chile, Valdivia, CL
(2) Instituto de Medicina Molecular Aplicada (IMMA), Facultad de Medicina, Universidad de San Pablo-CEU, Madrid, ES

Glioblastoma stem-like cells (GSCs) have been proposed as the main responsible of glioblastoma treatment failure due to its enhanced chemoresistance and tumorigenicity. Two GSCs subtypes have been described, proneural (PN) and mesenchymal (Mes), which have characteristic growth patterns and differential response to treatments. We have previously observed that adenosine signaling through its adenosine A3 receptor (A3AR) and A2B receptor (A2BAR) is increased in GSCs compared their differentiated counterpart, and its activation has been related to stemness-dependent chemoresistance. However, the role of adenosine signaling in PN and Mes subtypes has not been described, which is the aim of this study. Here, we identified PN and Mes GSCs from two patients and evaluate adenosine-dependent stemness regulation and chemoresistance, by spheres/colonies formation assays and MTS, respectively. Additionally, we have evaluated the β-catenin pathway as a possible mechanism of stemness regulation through adenosine. Here we show that Mes GSCs have enhanced extracellular adenosine accumulation and express higher A3AR, A2BAR, APCC and NT5E transcripts levels compared to PN GSCs. Pharmacological blockade of A3AR decreased spheres number and colony formation in both GSCs subtypes. A2BAR blockade but not A3AR, chemosensitized PN and Mes GSCs. The blockade of adenosine signaling in PN and Mes GSCs affected differentially the expression of β-catenin target genes and its nuclear translocation. In conclusion, adenosine signaling is active in both GSCs subtypes, thereby enhancing stemness through A3AR and promoting chemoresistance through A2BAR. Finally, adenosine signaling differentially regulated the activation of β-catenin pathway, suggesting a crosstalk between both signaling pathways on stemness regulation.

Fondecyt 1160777

Adenosine depletion reverts hypoxia-dependent invassiveness of Glioblastoma *stem-like* cells

Atenea Uribe-Ojeda[1], Ignacio Niechi[1], Erices José Ignacio[1], Francisco Rodas[1], Pamela Silva[1], Claudia Quezada[1].
(1) Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile

Glioblastoma is a grade IV astrocytoma considered as the most common and aggressive primary brain tumor. This is mainly due to a cell subpopulation with an extremly invasive potential called Glioblastoma Stem-like Cells (GSCs). This tumor presents hypoxic niches with elevated extracellular adenosine which activates signaling pathways related to HIF-2α expression/stability thereby promoting tumor malignancy. In others diseases, is possible to degrade adenosine using recombinant adenosine deaminase (ADA) to revert its phatological effects, thus, the aim of this study is to use ADA to degrade adenosine in order to decrease HIF-2α-mediated cell invasion in GSCs under hypoxia. GSCs were prepared from U87 human GBM cell line or human primary cultures using conditioned media. Cells were incubated under normoxic (21 % O_2) or hypoxic (0.5% O_2) conditions. Adenosine depletion was performed using 1 U/mL of recombinant ADA. mRNA and protein levels were measured by RT-qPCR and western blot, respectively. Protein stability was measured by cycloheximide assay and proteasome inhibition. Migration and invasion were measured by transwell and matrigel-coated transwell assay, respectively. HIF-2α protein levels but not mRNA expression decrease under ADA treatment in GSCs. HIF-2α protein degration is enhanced by treatment with ADA under normoxia and hypoxia in GSCs. Cell migration and invasion decrease in GSCs treated with ADA under hypoxia. In conlcusion, adenosine depletion in GSCs using adenosine deaminase decreases HIF-2α protein stability under hypoxia conditions, thereby decreasing cell invasiveness.

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Transcriptome characterization of intestines of adult zebrafish males fed soybean and fish meal protein-based diets

Luis Alfonso Valenzuela[1], Nicole Díaz[1], Sebastián Pacheco[1], Natalia Lam[1], Cristian Araneda[1], Pilar Ulloa[1].
(1) Laboratorio de Genética y Biotecnología en Acuicultura , Facultad de ciencias Agronómicas, Universidad de Chile, Santiago, CL

The limited availability of fishmeal in aquaculture has forced the industry to find alternative protein sources, and soybean meal (SBM) is commonly-used substitute. However, in cultured fish and zebrafish, SBM induces intestinal inflammation, interfering with health and growth. Under this scenario, main challenges for aquaculture are to successfully feed and cultivate herbivore diet-tolerant fish strains. Nowadays, it is possible the integration of nutrition and genomics analysis through nutrigenomics approach, helping to understand the effect of diet on gene expression. Our approach was to feed two populations with the same genetic background; one set of 19 families was fed with a control fishmeal diet (100FM) and the other set of 19 families was fed with soybean meal diet (50SBM). Males' intestine tissue from the 5% in both tails of the weight normal distribution from a population fed with 50SBM were selected (low and high growth; 50 mg vs 180 mg, respectively) to compare with males of average growth after feed with 100FM diet to carry out RNA-seq assays to evaluate transcriptomic differences. Statistical analysis showed 107 and 45 differentially expressed genes (DEGs) in low and high growth individuals respectively, regarding to control diet. In both cases, the top-ten Biological Process in which DEGs were involved were the same, including "primary metabolic process", "nitrogen compound metabolic process", "biosynthetic process", among others. The next step is evaluating the presence of SNPs within DEGs that may act as a potential biomarker to favor the selection of more herbivore diet-tolerant fish strains.

FONDECYT 11170847

Polymerization activity and cytotoxicity of molecules with affinity for LAU/PLA binding site of tubulin as novel stabilizing agents

Pilar Vásquez[1], Matías Zúñiga[2], Leonardo Guzmán[1], Verónica Jiménez[3].
(1) Departamento de Fisiología, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, CL
(2) Center for Bioinformatics, Simulations and Modelling, Facultad de Ingeniería, Universidad de Talca, Talca, CL
(3) Departamento de Ciencias Químicas, Facultad de Ciencias Exactas, Universidad Andrés Bello, Concepcion, CL

The importance of microtubules in cellular division set these proteins as pharmacological targets for antimitotic agents, known as tubulin binding agents (TBA), which can promote stabilization or destabilization of tubulin polymerization. The occurrence of adverse drug reaction associated to several of these agents drives the need for the development of new TBAs with a safer pharmacological profile. In this regard, a combination of computational virtual screening, molecular dynamics and binding free energy estimations was performed by our group, based on the stabilizing LAU/PLA binding site of tubulin. A set of 7 candidates were proposed as potential stabilizing agents with affinity for the site. In this work, we confirm the polymerization capacity for these 7 candidates in vitro at concentrations of 50 and 100 µM. Also, we observed an additive effect of the compounds when co-treating with Taxol, confirming a non-competitive binding with taxane-site binders. Finally, viability assays in a cancer cell line were developed showing a cytotoxic effect of molecules at 100 µM. These results set a starting point of further studies for the characterization of the novel agents that will open possibilities for the rational screening of new tubulin stabilizing agents.

FONDECYT 1160060 and 1170853

Molecular dynamics simulations provide insights into the enzyme-substrate complex structure of human Arginase I

José Luís Velázquez-Libera[2], Hernández-Rodríguez, Erix W.[1], Julio Caballero[2].
(1) Escuela de Química y Farmacia, Facultad de Medicina, Universidad Católica del Maule, Talca, CL
(2) CBSM, Facultad de Ingeniería, Universidad de Talca, Talca, CL

The human Arginase I (hARGI) enzyme hydrolyzes L-arginine into L-ornithine and urea, during the last urea cycle reaction. The extrahepatic overexpression of hARGI is associated with several cardiovascular-related diseases [1]. Therefore, hARGI can be an interesting therapeutic target to treat heart and blood vessel pathologies, considering besides an expected tolerance to keep an appropriate ammonia detoxification rate due to the high concentration of this enzyme in the liver [2]. However, the design of new and stronger inhibitors affecting the hARGI activity implies a better understanding of the enzyme-substrate (ES) complex structure to get insights into the catalytic mechanism of hARGI, which remains unclear. In fact, ABH-like inhibitors mimic tetrahedral intermediate described by two proposed mechanisms for arginase catalysis [3], [4]. Here, we investigate six representative ES complexes for both variants of the Mn(II)-coordinated nucleophile and the electric charge of the L-arginine at the active site, using molecular dynamics (MD) simulations. Three productive 100ns runs were performed under NPT ensemble at 300 K within the AMBER suite [5], applying its own force field. Our calculations reveal that the more favorable ES complex structure contains hydroxide ion nucleophile and a positively charged arginine into the active site at reaction pH.

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Development and characterization of a biocompatible photosynthetic oxygenation solution to preserve organs for transplantation

Valentina Veloso[1], José Tomás Egaña[1], Rolando Rebolledo[1,2].
(1) Institute for Biological and Medical Engineering, Schools of Engineering, Medicine and Biological Sciences, Pontifical Catholic University of Chile, Santiago, CL
(2) Surgical Service, Sótero del Río Hospital, Santiago, CL

Introduction:
The global shortage of donor organs for transplantation remains a major healthcare challenge. It is crucial to improve the current techniques of graft preservation to decrease the discard rate. Oxygenation during preservation period is a key factor to minimize the hypoxia injury and maintain the organ viability until the transplantation. In recent years, our research group has proposed a new strategy for tissue oxygenation based on photosynthesis performed by microorganisms. In this context, the aim of this work is to develop a biocompatible photosynthetic oxygenation solution with microalgae to oxygenate isolated organs for transplantation.

Methodology:
Microalgae *Chlamydomonas reinhardtii* were incubated during 0-24h in: 1) Control (TAP growth media); 2) RLM (Lactated´s Ringer solution supplemented with mannitol); 3) 50% (Mixed solution 1:1 TAP: RLM). Viability of microalgae was assessed by growth in agar plates, cell counting and cytometry (live/dead dyes). Oxygen production rate was measured by oxygraphy. The biocompatibility of the photoshyntetic solution (RLM + microalgae) was evaluated in zebrafish larvae as a toxicological model.

Results:
Both viability and photosynthetic oxygen production rate of microalgae were not affected after 24h incubation in RLM nor 50% solution compared to control. This suggests that the photosynthetic solution is viable and releases oxygen in response to light. Furthermore, zebrafish larvae did not show signs of toxicity after 24h exposure to the photosynthetic solution.

Conclusions:
We demonstrate that microalgae remain viable in an organ perfusion solution. The resulting photosynthetic solution can produce oxygen in a light-responsive manner and it is biocompatible *in vivo*.

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Palmitic acid reduces mitophagy via primary cilia in the hypothalamic neuronal cell line N43/5

Leslye Venegas[1], Ana Batista-González[2], Valentina Parra[1], Alfredo Criollo[2].
(1) Bioquimica y Biologia Molecular, Ciencias Quimicas y Farmaceuticas, Universidad de Chile, Santiago, CL
(2) Instituto de investigación en Ciencias Odontológicas, Odontologia, Universidad de Chile, Santiago, CL

Introduction:
Saturated fatty acids (satFA) are an important factor in the increased global obesity epidemic. Studies in obese mice shows that satFA are high in the brain and, in particular, palmitic acid (PA) disrupt the hypothalamic function and severely impact POMC neurons, which play an important role in the feeding behavior. Some studies show that POMC neurons under satFA exposure, present an abnormal mitochondrial morphology with irregular sizes and a fragmented pattern; in addition to a blockage of the autophagic flux. Moreover, mice fed under a high fat diet present a decreased length, area and volume of the primary cilia (PC) in the hypothalamus, organelle which participates in metabolism and autophagy regulation. In addition, *in vitro* and *in vivo* ciliophaties models, show abnormal mitochondrial morphology. The aim of this study was to determine if PA alter the PC, and if this is due to mitochondrial disfunction in the POMC neurons.

Methodology:
We used the hypothalamic cell line N43/5, under two conditions: BSA (control) or PA (100 µM) and analyzed the percentage of ciliated cells and mitochondrial morphology using confocal microscopy and mitophagy markers by means of Western blot.

Results:
PA reduced the percentage of ciliated cells and induced mitochondrial fragmentation, as well as triggered mitophagy blockade. Interestingly, these effects were modulated in cells with diminished PC and later treated with PA.

Conclusions:
Our results suggest that PA blocks the mitophagic process partly via primary cilia in the N43/5 cells.
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Molecular determinants of the TRPC6 channel association with VAPA endoplasmic reticulum contact proteins

Ariela Vergara-Jaque[1,2], Alhejandra Alvarez[2,3], Javier Díaz[1], Horacio Poblete[1,2], Wendy González[1,2], Ian Silva[2,3], Boris Lavanderos[2,3], Oscar Cerda[2,3].
(1) Center for Bioinformatics, Simulation and Modeling, Facultad de Ingeniería, Universidad de Talca, Talca, CL
(2) Millennium Nucleus of Ion Channels-Associated Diseases (MiNICAD), CL
(3) Programa de Biología Celular y Molecular, Instituto de Ciencias Biomédicas (ICBM), Facultad de Medicina, Universidad de Chile, Santiago, CL

Important advances have been performed to understand the mechanisms that regulate the activity of TRPC channels. Protein-protein interactions have recently emerged as promising pharmacological targets. Here, we evaluate the association of the vesicle-associated membrane protein-associated protein A (VAPA) and the transient receptor potential cation 6 channel (TRPC6). VAPA is an endoplasmic reticulum resident protein involved in vesicle trafficking, membrane fusion, protein complex assembly, and cell motility. Through a mass spectrometry-based proteomics approach, we identified VAPA as a novel TRPC6-interacting protein. In addition, TRPC6-VAPA association was validated by co-immunoprecipitation assays in HEK293 cells. In humans, VAPA has shown to bind proteins containing a FFAT motif essential for its function. Interestingly, TRPC6 exhibits a putative FFAT motif that might mediate the binding with VAPA. To characterize this interaction, we refined the recently revealed TRPC6 crystal structure adding missing residues surrounding the putative FFAT motif. Protein-protein docking were then carried out with the VAPA structure, identifying as contact region a set of residues previously found in the binding interface between VAPA and other FFAT-containing proteins. MD simulation of the TRPC6:VAPA complex were carried to describe the specific interactions modulating that association. The trajectories reveal polar contacts between negative-charged residues surrounding the putative FFAT motif in TRPC6 and positive-charged residues in VAPA. We hypothesize that this interaction constitutes a mechanism to regulate the TRPC6 trafficking to plasma membrane, serving VAPA as a novel TRPC6-regulatory protein. To our knowledge, this is the first study reporting TRPC channels to be modulated by endoplasmic reticulum contact proteins.

FONDECYT #11170223 and MiNICAD

Investigating TET proteins partners that control hydroxymetylated genes in the adaptive immune system

Daniela Villalobos[1], Noelia Blanco[1], Cristina Silva[1], Marcela Sjoberg[1].
(1) Biología Celular y Molecular, Ciencias Biológicas, Pontificia Universidad Católica de Chile

Ten-eleven translocation (TET) enzymes belong to a family of DNA dioxygenases with catalytic activity towards the 5-methylcytosine mark (5mC), an epigenetic modification essential for the regulation of gene expression and the maintenance of cellular identity. TET proteins can turn 5mC into 5-hydroxymethylcytosine (5hmC) a modified base that accumulates in embryonic stem (ES) cells, neurons in the CNS and immune cells. The association of TET proteins with epigenetic regulators has been described in different cellular models. One of these regulators is the enzyme O-linked *N*-acetylglucosamine (O-GlcNAc) transferase (OGT), which directly binds and regulates TET protein phosphorylation, enhancing its activity. Previous work of the laboratory identified a set of 5hmC marked genes in $CD4^+$T lymphocytes. To better understand the establishment and readout of the 5hmC mark over this set of genes, we used splenic $CD4^+$T lymphocytes from C57BL/6 mice to evaluate the expression and subcellular localization of TET proteins. We then interrogated the interaction between TETs proteins and OGT by co-immunoprecipitation (co-IP) and finally we asked if TET and OGT proteins are recruited to the promoter regions of 5hmC marked genes by chromatin immunoprecipitation (ChIP-qPCR). Identifying TET binding partners like OGT, is key to comprehend how the cellular metabolic state and external stimuli may be controlling the gene expression status of hydroxymethylated genes in $CD4^+$T lymphocytes.

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Effect of pollen storage temperature on its viability in blueberries (*Vaccinium corymbosum* L.)

Mauricio Vivar[1], Amanda Donoso[1], Loreto Prat[1], Carlos Muñoz[1].
(1) Producción Agrícola, Laboratorio de Mejoramiento Genético de arándanos, Ciencias Agronómicas, Universidad de Chile, Santiago, CL

In plant breeding, pollen quality is important for success in targeted crossings. The use of recently collected pollen is the crossing preferred method, but due to differences in the timing of anthesis among cultivars; pollen is often stored for use in the following season. In this study, the effect of different storage temperatures on the viability of pollen of 10 segregants was evaluated. To do this, pollen was obtained from flowers collected at anthesis and stored in Eppendorf tubes at 24°C, -20°C and -80°C for up to 120 days. At the time of collection and every 30 days, viability was evaluated by germinating pollen over a solid culture medium. Percent germination was determined by observing pollen tube emergence under a microscope with a 10x magnification. Results indicate that, in general, refrigerated storage produced a decrease in pollen fertility in all segregants after 120 days of storage, compared to % germination at the time of collection. When pollen was stored at -20°C, a decrease in % germination was observed, that varied between 87 and 3%, depending on the segregante. When the storage temperature was -80°C, germination varied between 64 and 3%. This indicates that there is genetic variability for pollen resistance to low temperatures and that, the lower the storage temperature, the greater the decrease in pollen fertility.

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Four amino acids define the CO_2 binding pocket of enoyl-CoA carboxylases/reductases

David Saez San Martin[1], **Esteban Vöhringer-Martinez[1]**, Gabriele Stoffel[2], Tobias Erb[2], Soichi Wakatsuki[3], Bastian Vögeli[2].
(1) Departamento de Físico-Química, Facultad de Ciencias Químicas, Universidad de Concepción, Concepción, CL
(2) Department of Biochemistry and Synthetic Metabolism, Max Planck Institutte for terrestrial Micobiology, Marburg, DE
(3) SLAC National Accelerator Laboratory, US

Carboxylases are biocatalysts that capture and convert carbon dioxide (CO2) under mild conditions and atmospheric concentrations at a scale of more than 100 Gt annually.(1) However, how these enzymes bind and control the gaseous CO2 molecule during catalysis is only poorly understood. One of the most efficient classes of carboxylating enzymes are enoyl-CoA carboxylases/reductases (Ecrs), which outcompete the plant enzyme RubisCO in catalytic efficiency and fidelity by more than an order of magnitude. Here we investigated from an in silico perspective the interactions of CO2 within the active site of Ecr from Kitasatospora setae. Using experimental data and performing advanced computer simulations we show that four amino acids, N81, F170, E171 and H365 are required to create a highly efficient CO2-fixing enzyme.(2) Together, these four residues anchor and position the CO2 molecule for the attack by a reactive enolate created during the catalytic cycle. Altogether our study reveals unprecedented molecular details of selective CO2 binding and C-C bond formation during the catalytic cycle of nature's most efficient CO2-fixing enzyme. This knowledge provides the basis for the future development of novel catalytic frameworks for the capture and conversion of CO2 in biology and chemistry.

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