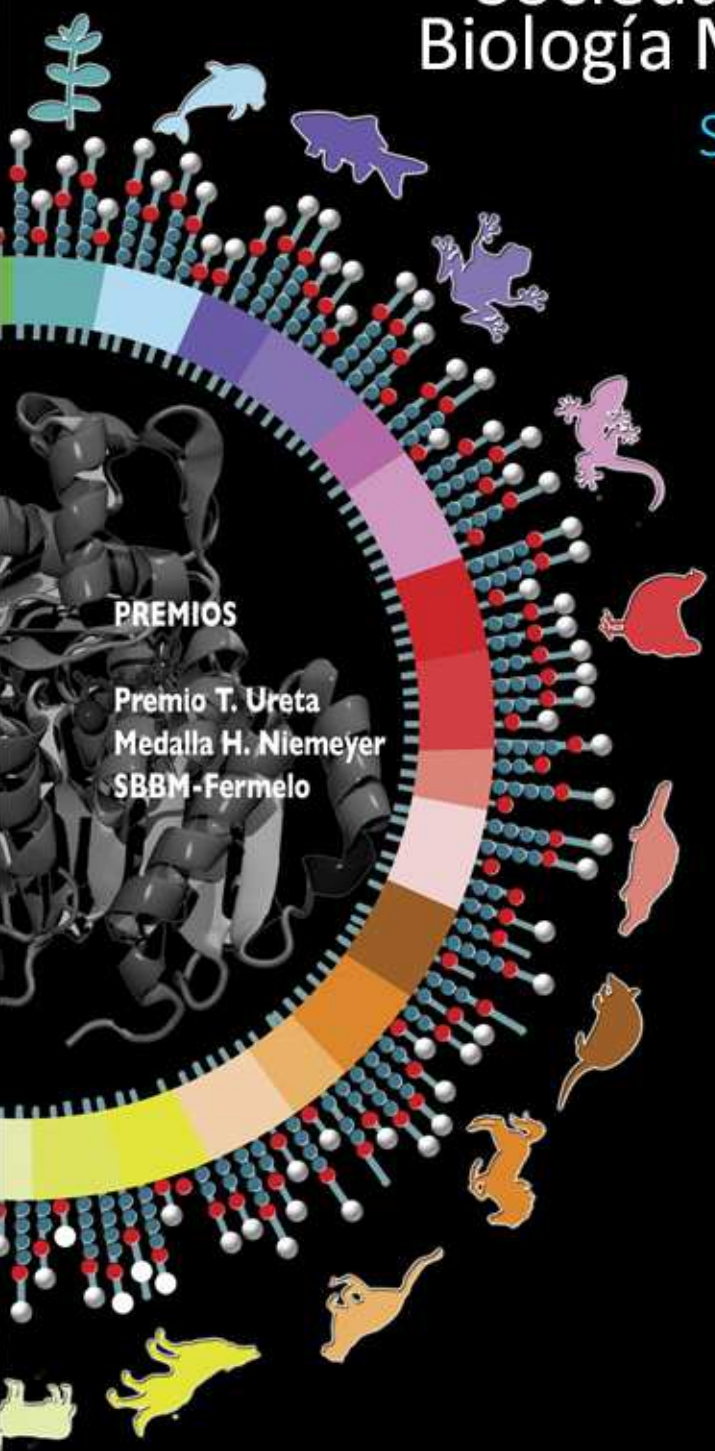
XXXVII Annual Meeting Sociedad de Bioquímica y Biología Molecular de Chile

September 30 – October 04, 2014

Hotel Dreams, Los Volcanes, Puerto Varas



Conferencias

Inaugural
Dr. Tom Beeckman, Bélgica

Plenaria
Dr. Joaquín Espinosa, Argentina

Severo Ochoa
Dra. Encarnación Martínez, España

Osvaldo Cori
Dra. Jenny Fiedler, Chile

PABMB
Dr. Grabriel Rabinovich, Argentina

Diálogo con la Ciencia
Dr. Mario Pino, Chile

Simposios

Synthetic Biology and Optogenetics

Cell Signaling and Disease

Bioinformatics

Microfluidics in Quantitative Biology

Protein Cristallography Made in Chile

Understanding Protein Function and Structure: From Ensemble to Single Molecule Approaches

Informaciones y contacto:
www.sbbmch.cl
Secretario: Dr. Andrew Quest
secretariasbbm@gmail.com Fono 56-2-2978 6371

XXXVII Annual Meeting
Sociedad de Bioquímica y Biología Molecular de Chile

September 30 – October 04, 2014

Puerto Varas

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LECTURES

Opening Lecture

A molecular blueprint for the Arabidopsis root system

Beeckman, T[1]., [1]Department of Plant Systems Biology - VIB, Sciences, Ghent University.

Plant roots serve a multitude of functions. They anchor and supply plants with water and nutrients and exchange various growth substances with the shoots. Understanding how root systems develop is crucial for maximizing crop production in a world in which population is increasing and the amount of arable land is decreasing. Lateral root formation or root branching is determinative of root-system architecture. During root branching, a rigorous coordination of developmental and environmental control mechanisms on cell proliferation and differentiation are taking place. In order of appearance, initially a group of founder cells becomes specified followed by a renewal of mitotic activity, asymmetric division and a concurrent start-up of differentiation processes. The signaling cascades towards the specification of the lateral root founder cells and the asymmetric cell divisions are up to now not elucidated and represent the major theme of our current research. The accessibility of this developmental process in Arabidopsis, the availability of adequate tools and the possibility to experimentally induce the course events has put us in a privileged position to unravel very fundamental aspects of growth and development in a multi-cellular context. The most recent progress in our understanding of root branching will be discussed.

Plenary Lecture

Taming the p53 network for therapeutic purposes

Espinosa, J[1]., [1]Molecular, Cellular and Developmental Biology University of Colorado at Boulder.
p53 is the most commonly inactivated tumor suppressor gene in human cancer. The p53 gene network is composed of functionally distinct gene modules mediating diverse cellular responses to stress including cell cycle arrest, senescence, apoptosis and autophagy. The molecular mechanisms defining how cells adopt a specific response upon p53 activation are poorly understood, which hampers the development of therapies harnessing the apoptotic potential of p53 for selective elimination of cancer cells. Why do some cell types survive whereas others die upon p53 activation?

Several projects in our lab investigate how pleiotropy is generated within the p53 transcriptional program and how the network can be manipulated to produce specific cellular responses upon p53 activation. We performed mechanistic studies using global measurements of nascent RNA synthesis (GRO-seq), steady state RNA levels (microarray gene profiling) and p53 occupancy (ChIP-seq) to demonstrate how the p53 transcriptional program is qualified at the transcriptional and post-transcriptional levels. We have also performed genome wide shRNA screens to identify signaling pathways that control the cellular response to p53 activation. Finally, we employed this knowledge to improve the therapeutic efficacy of p53-based targeted therapies currently being tested in clinical trials for the treatment of various cancers.

Diálogos con la Ciencia

The first settlers in Chile: Monteverde and Pilauco sites

Mario Pino. Facultad de Ciencias, Universidad Austral de Chile, Independencia 631, Valdivia.

In 1932 the discovery of the Clovis site in New Mexico provided the basis for proposing that all Americans are descended from these megafauna hunters Tens of Clovis sites have been dated between 11,050 to 10,800 ^{14} C yr BP. The archaeological site of Monte Verde (30º 41' 14.7 "S, 73˚ 12 '22.2''W), was discovered by local farmers in 1976. From 1978 until today the site has been excavated, analyzed and interpreted. Plant materials and bones of megafauna have been dated to 12,500 ^{14}C yr BP.

Since Monte Verde is located in the south of Chile, the difference of 1500 ^{14}C yr BP between Clovis and Monte Verde is not real. It is necessary to add the time that took the human groups trip from northern of North America to southern Chile, which means that the ancestors of the inhabitants of Monte Verde entered North America about 20,000 years ago. But Monte Verde is not only to this day, the oldest archaeological site in the Americas. It is a well-preserved site, consisting of a "toldo" and a special area. The quality of preservation is related to flooding on the site, probably due to an increase in groundwater, that transform a bar in a creek (with the camp) into an anoxic wetland. For this reason a layer of peat sealed the site. In Monte Verde a human footprint, and remains of bones and meat of gomphothere, ropes, knots and a range of edible and medicinal plants and algae are recorded, including the oldest American wild potato. The Pilauco site (40˚34 '05 "S, 73˚70'10W) was discovered in 1986 by workers of a construction company. It is set in the city of Osorno. It has been scientifically analized since 2007. The carrier layer deposited in a wetland is dated between 14,300 and 12,500 ^{14}C yr BP and includes fossils gomphotheres, horse, camel, bear, xenartra, pudú, skunk, coipo, plus insects, hair, coprolites, parasites and a human footprint. Archaeological artifacts associated to gomphothere bones reveal allochthonous origin of the raw lithic materials in old Tertiary volcanoes at the Coastal Range.

If Monte Verde is a camp, Pilauco site is a place to obtaine meat. Both confirm that the peopling of the Americas occurred at very early times of the late Pleistocene.

Osvaldo Cori Lecture

The Ying-Yang od stress: effect on neural circuit remodeling

Fiedler, J[1]., [1]Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad De Chile.
(Sponsored by FONDECYT 1120528.)

The stress response involves a myriad of effects promoting the activation of nervous system and release of adrenal glucocorticoids (GCs). These hormones are potent modulators of cognitive processes, such as learning, memory, and recall. Corticoid secretion induced by acute stress affects positively or negatively the memory processes. Besides, stress-induced GCs secretion during the learning of a new task has been critically involved in memory consolidation. This phenomenon is rooted in brain regions targeted by corticoids to integrate the physiological and behavioral responses during stress and adaptation to subsequent stressful events. In contrast to acute stress, the repeated exposure to stressors may result in abnormal changes in brain plasticity that impairs the ability to respond properly to subsequent stressors and also reduces cognitive abilities. Further, it is not yet clear how adaptive mechanisms triggered by acute stress are modified by chronic stress producing maladaptive responses. The positive and negative effects of GCs on memory process can be related to local changes in the excitatory glutamatergic synapses involving variation in the number of synapse, receptor trafficking and local regulation of translation of mRNA in the synapse. The knowledge of stress effect on brain may constitute the bases to understand the mechanisms associated to the susceptibility to stress-related disorders.

Severo Ochoa Lecture

RNA-binding proteins controlling internal initiation of translation in RNA viruses

Martinez-Salas, E[1]., [1]Genome Dynamics and Function Centro de Biologia Molecular Severo Ochoa, CSIC-UAM, Madrid, Spain.

Internal ribosome entry site (IRES) elements govern protein synthesis in a wide variety of RNA viruses. Recruitment of the translation machinery to the viral RNA requires the interaction of the IRES element with RNA-binding proteins, termed ITAFs. Novel ITAFs were identified in riboproteomic approaches carried out with two IRESs, hepatitis C virus and foot-and-mouth disease virus. Among other factors, the cytoplasmic protein Gemin5, a factor involved in the biogenesis of small nuclear ribonucleoproteins, was bound to both IRESs. Interestingly, Gemin5 is targeted by viral proteases in infected cells and behaves as a negative regulator of translation. Analysis of this protein showed that the C-terminal region bears two noncanonical bipartite RNA-binding sites (RBS1, RBS2). The three-dimensional structure of RBS1 shows a flexible conformation rather than a defined tertiary structure and exhibits greater affinity for RNA than RBS2. However, RBS2 harbors the IRES repressor domain. Comparison of the RNA-binding capacity and translation control properties of Gemin5 polypeptides to the proteolysis products observed in infected cells reveals that non-repressive products accumulates during infection while the repressor polypeptide is not stable. Biochemical and structural characterization of Gemin5 and other ITAFs will be discussed to illustrate the IRES-driven translation initiation process.

PABMB Lecture

Regulatory circuits mediated by lectin-glycan interactions in chronic inflammation and cancer

Gabriel A. Rabinovich, Laboratory of Immunopathology, Institute of Biology and Experimental Medicine (IBYME, CONICET) and Laboratory of Structural and Functional Glycomics, Department of Chemical Biology, Faculty of Exact and Natural Sciences, University of Buenos Aires, 1428 Buenos Aires, Argentina. E-mail: gabyrabi@gmail.com

The responsibility for deciphering the biological information encoded by the glycome is assigned to endogenous glycan-binding proteins or lectin whose expression is regulated at sites of inflammation and tumor growth. With the overarching goal of generating more rational therapeutic strategies, our laboratory investigates the molecular interactions between endogenous lectins and cell surface glycans leading to the control of immune tolerance and angiogenesis. In the past years we identified an essential role for galectin-1, an endogenous lectin with specificity for poly-N-acetyllactosamine-enriched glycans in promoting tumor-immune escape and regulating chronic inflammation. Investigation of the mechanisms underlying these effects revealed the ability of this lectin to selectively eliminate Th1 and Th17 cell subsets, instructing the differentiation of tolerogenic dendritic cells (DCs) and modulating astrocyte-microglia interactions. In the presence of galectin-1, DCs acquire an interleukin 27-dependent regulatory function and microglial cells are polarized toward an M2-type pro-resolving phenotype. More recently, our laboratory found that interactions between galectin-1 and complex N-glycans on VEGFR2 can link tumor hypoxia, endothelial cell signaling, angiogenesis and tumor inflammation. These results highlight the central role of the galectin-1-glycan axis in immunoregulatory and vascular signaling programs during inflammation and tumor growth.

Symposia

Synthetic Biology and Optogenetics Symposium

Synthesizing the Rules for Expression: Understanding the Behavior of Genetic Elements using Synthetic DNA libraries

Goodman, D[1]., Church, G[1].,Kosuri, S[1,2].,[1]Genetics Harvard Medical School.[2]Biochemistry University of California, Los Angeles.

The unpredictability of gene expression hinders our ability to engineer biological systems. The majority of our knowledge of gene expression has come either from measuring the activity of natural genes or by perturbing genetic systems at relatively small scales. We still have a limited understanding of how altering genetic elements affects gene expression and how to quantitatively tune expression for biotechnological applications. Utilizing large libraries of custom synthetic DNA oligonucleotides and multiplex reporter assays, we tested the behavior and composability of over 12,000 genetic elements that control transcription and translation in *Escherichia coli* and quantified their interactions as well as global effects. We additionally examined how the use of synonymous codons at the N-terminus of genes influences gene expression, and found that using rare codons instead of common ones strongly increased expression. We demonstrated that reduced mRNA secondary structure and not codon rarity is responsible for expression increases. The ease and scale of our approach allows us to quantify complex natural phenomena and makes it feasible to screen large libraries of synthetic DNA elements for desired behavior.

Improving Optogenetic Devices: Using Chemistry and Structural Mechanisms to Provide Tunable Platforms for Light-Controlled Protein:Protein Interactions.

Zoltowski, B[1]., [1]Chemistry, Assistant Professor, Southern Methodist University.

Several platforms have been employed to yield optogenetic devices. Unfortunately, extending these toolsets to broader applications has proven difficult. What we need is a light-sensitive protein module that can easily be coupled to a diverse array of signaling components. Light-Oxygen-Voltage domains are small, light-sensitive modules that fit these parameters, however harnessing the light-activation pathways in a robust manner has been limited to select systems. The use of LOV-based optogenetic tools can be improved by deciphering three elements: 1) The precise signaling mechanism coupling LOV photochemistry to signaling modules. 2) Improving LOV protein stability and 3) Precise tuning of the spectral and temporal properties of the protein-systems without affecting the output signal. Several methods have been used to tune the spectral properties of LOV domains, however they often neglect the impact of these photocycle-altering variants on protein stability and signaling mechanisms. Our lab has identified several sites that allow for tunability of both photocycle properties and improved protein stability. Our systematic study of the effects of these variants on photocycle-lifetime, spectral sensitivity, protein stability, and fidelity and direction of the output signal will allow for construction improved optogenetic tools.

Optogenetic Control of Signalling Processes in Mammalian Cells

Weber, W[1]., [1]Biology II/Biochemistry/Synthetic Biologie, Biology, University of Freiburg.

Synthetic Biology aims at the design and implementation of biological systems with desired properties using a modular approach based on well-characterized biological building blocks. In order to achieve optimal spatio-temporal resolution in controlling the function of the synthetic biological system, optogenetic tools have been developed that allow controlling molecular events along the whole signal transduction pathway.

In this lecture we will report our work on engineering signaling processes in mammalian cells at the following levels: control of cell fate and function by optically modulating the extracellular matrix of cells; controlling map kinase signaling by engineering blue light-responsive kinases; controlling nuclear translocation by functionally reconstructing the phytochrome-mediated nuclear transport from plants in mammalian cells and finally independently controlling multiple promoters within one single cell by engineering orthogonal multichromatic transcription factors.

This work on the development and application of optical tools will be complemented by metabolic engineering strategies for producing light-sensing chromophores in mammalian cells.

Natural optogenetic circuits in fungi: from basic biology to the development of tools for synthetic biology.

Larrondo, L[1]., [1]Millennium Nucleus for Fungal Integrative and Synthetic Biology and Departamento Genética Molecular y Microbiología, Facultad de Ciencias Biologicas, Pontificia Universidad Católica De Chile.

The fungus *Neurospora crassa* is an important model for the understanding of photobiology and circadian regulation. The proteins White Collar-1 (WC-1) and White Collar 2 (WC-2) form a transcriptional complex that, in the presence of blue light, can lead to strong activation of their target genes. The ability to sense light relays on the presence in WC-1 of a LOV (Light Oxygen Voltage) domain. After continuous exposure to light WCC activity decreases due to photoadaptation, which is mediated by another LOV containing protein, called VVD. These components are also part of the basic circuitry of the Neurospora circadian oscillator. The latter also involves the protein FREQUENCY, which in addition to WCC forms a transcription translational negative feedback loop (TTFL), in which WCC drives the expression of *frq*, while this gene product inhibits its own synthesis. These oscillations are autonomous and can be entrained to environmental cues, such as light. Through different approaches we are exploring the impact of light regulation on gene expression, further dissecting the molecular mechanisms involved. In addition, we are testing the modularity of some of these light-sensing components and their transferability and properties as orthogonal genetic switches. Finally, we are rewiring central components of both light- and circadian mechanisms, analyzing the properties of “hybrid synthetic oscillators” though real-time reporters with high temporal resolution. FONDECYT 1131030, MN-FISB NC120043.

Protein Cristallography Made in Chile Symposium

Protein Crystallography made in Chile, Snapshots.

Bunster, M[1]., [1]Depto. Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.

2014, The International Year of Crystallography declared by the United Nations, celebrates the centennial of the Nobel Prize of Max Von Laue, the first scientist to diffract x-rays with a crystal. This event emphasizes worldwide the contribution of Crystallography to the development of science and technology. Protein Crystallography, began when Perutz used the diffraction of X-rays produced by crystals of hemoglobin to solve its molecular structure. Crystallography in Chile began at the Physics Department of the Universidad de Chile; the use of X-ray diffraction to analyze biological membranes and fibers was developed at the Universidad de Concepción and the Universidad Austral de Chile during late 1960 and 1970. Protein Crystallography had its early development at the Universidad de Concepción in 1980. The importance of Protein Crystallography is well recognized in the scientific community, as it is the need to increase the critical mass in Chile. Proyecto FONDECYT Nº113.0256.

X-ray crystallography: a tool to understand enzyme function and a perspective on the establishment of local facilities

Cabrera, R[1]., [1]Departamento de Biologia, Facultad de Ciencias , Universidad De Chile.

The structural characterization through X-ray crystallography has become the cornerstone to understand protein function and evolution. In Chile, this is a developing area that could greatly contribute to the generation of knowledge in important fields such as cell biology, biotechnology and medicine.

I will first analyze how different structures obtained by X-ray crystallography have guided the understanding of the phenomenon of substrate inhibition by ATP of the *E. coli* phosphofructokinase-2 (Pfk-2). This inhibition impedes the futile hydrolysis of ATP when the bacterium is feeding over gluconeogenic sources. Different crystallographic structures have allowed the description of the inhibited form of the enzyme, the allosteric binding site, and the conformational changes associated to the binding of substrates and products. Also, structural comparisons of Pfk-2 to its evolutionary relatives in the ribokinase family, and to the evolutionarily unrelated Pfk-1, illustrate events of divergence and convergence of structure and function.

For all this work, obtaining and processing the X-ray data was possible only trough the collaboration with Brazilian crystallographers. Furthermore, after more than 20 years of protein crystallography in Brazil, some aspects could be analyzed in regard to the path in which Chile is paving the way towards installing local X-ray facilities.

ACT1107, EQM120208

How we decipher protein sorting signal-recognition from Southern Chile

Mardones, G[1]., Ross, B[1].,Corales, E[1]., Lin, Y[1].,Arriagada, C[1].,Cavieres, V[1].,Burgos, P[1].,[1]Department of Physiology, School of Medicine, Universidad Austral de Chile. (Supported By FONDECYT-1130710, And DID-UACh.)

To achieve their functions, proteins establish a series of interactions with other molecules and macromolecules. In our laboratory, we are interested in the structural bases of the interactions that underlie the recognition of signals contained in transmembrane proteins that use the secretory pathway to reach their final destination. These intracellular transport processes rely in cytosolic machineries that decode structural information in cargo proteins. The resulting interactions trigger the formation of membrane-bound transport-carriers known as vesicles. A family of cytosolic adaptor protein (AP) complexes mediates sorting of cargo proteins to endosomes, lysosomes, and specific domains of the plasma membrane of polarized cells. This is mediated by recognition of tyrosine-based signals fitting the YXXØ motif (where Ø is an amino acid with a bulky hydrophobic side chain). During the last few years we have put effort on the characterization of some of these interactions using methodologies that include X-ray crystallography. These studies led to the discovery of a distinct form of sorting signal recognition. In my talk I will give an account of some of our findings, and of the challenges of establishing a lab that uses macromolecular X-ray crystallography, but in the southern part of Chile.

Crystallographic studies on Phycobiliproteins from *Gracilaria chilensis*

Martinez, J[1]., [1]Bioquimica y Biologia Molecular, Ciencias Biologicas, Universidad De Concepción.

The Phycobilisomes from *Gracilaria chilensis* has been studied to understand the light transfer process in the light harvesting for photosynthesis, essential for algae and cianobacteria.

Phycoerythrin, Phycocyanin and Allophycocyanin are the principal chromophorylated protein in this macromolecular assembly.

We solved the crystal structure of Phycoerythrin from merohedral twinning crystals that diffracted at 2.2 Å resolution at the ESFR synchrotron. To solve the twinning and the refinement we used SHELX97 program. The phase problem was solved using AMORE program. The final model quality was assessed by PROCHECK and WHAT IF. The final model has 5151 atoms for 4 chains and 448 atoms for 10 chromophores. The R-Phycoerythrin is a hexamer of a heterodimer. This was the first protein structure solved and deposited from Chile.

The structure of Phycocyanin from monoclinic crystals that diffracted at 2.0 Å at the IMCA-CAT Synchrotron was solved by molecular replacement using CNS program. The final model has 15.024 atoms and is a hexamer with 12 subunits with 18 chromophores. The coordinates and structure factor of both structures were deposited in PDB under the code 1 EYX and 2BV8.

Both structures have allowed us to predict preferential light pathways across the phycobilisomes.

Funded by FONDECYT 113.0256

Understanding Protein Function and Structure: From Ensemble to Single Molecule Approaches

Mapping solvent accessibility in proteins. Insights into folding and interactions

Delfino, J[1].,Bernar, E[1].,Gomez, G[1].,[1]Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. (Sponsored by ANPCyT, UBACyT, CONICET)

Topography of proteins and their interactions can be investigated through photochemical mimicry of the aqueous solvent, an approach aimed at estimating the size and nature of the solvent accessible surface area (SASA). After reacting diazirine (DZN, the smallest CNN heterocycle) with proteins, it is possible to measure quantitatively the extent of modification (methylation) by the use of radiotracers (tritiated DZN), by metrics derived from modern mass spectrometry techniques (MALDI-TOF and ESI-MS) or by multidimensional NMR. Maximal resolution of the labeled site is achieved after fragmentation into small peptides or individual amino acids. Interestingly, the NMR approach does not demand cleavage and is potentially rich in conformational information. Predictably, methylation of amino acid side chains rules the DZN modification phenomenon, giving rise predominantly to insertions into CH bonds. Thus, the probability of reaction at individual sites along the polypeptide reveals the map of solvent accessibility. Conformations can be distinguished corresponding to native or intermediate states, or the unfolded ensemble. Moreover, a paradigm of a peptide-protein complex (calmodulin-melittin) illustrates the value of this approach as a foot-printing technique able to pinpoint the area of interaction. One cannot overemphasize the worth of these new methods for the benefit of structural proteomics and interactomics.

Capturing protein structure ensembles at high resolution using hydrogen-deuterium exchange mass spectrometry

Ramírez-Sarmiento, C,A[3]., Baez, M[1].,Wilson, C[1]., Balasubramaniam, D[2].,Villalobos, P[3].,Babul, J[3].,Komives, E[2]., Guixé, V[3].,[1]Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.[2]Department of Chemistry and Biochemistry University of California San Diego.[3]Departamento de Biología, Facultad de Ciencias, Universidad de Chile. (Funding: FONDECYT 1110137)

Although our knowledge about protein folding and protein interactions has dramatically increased over time, capturing the local structural changes experienced by a given protein during folding and binding remains as a challenge. The flexibility of a protein structure can be determined using hydrogen-deuterium exchange, which measures the solvent accessibility of backbone amide protons. Then, protein fragmentation by proteolysis and analysis of the resulting peptides by mass spectrometry allows sampling of the structural heterogeneity of local regions of the protein over time. Using the dimeric enzyme phosphofructokinase-2 (Pfk-2) from *Escherichia coli* as a model, we used hydrogen-deuterium exchange mass spectrometry (HXMS) to determine the local structural changes occurring during its cold denaturation. While the native-state of dimeric Pfk-2 described by HXMS fully reconstructs the structure solved by X-ray crystallography, the cold-denatured state appears to form by solvent penetration throughout the structure and concurrent dissociation into monomers, with a kinetic unfolding rate of 1×10^{-4} s^{-1}. Comparison of the native-state ensemble of Pfk-2 with the cold-denatured state, the monomeric mutant L93A and the intermediate state seen by GndHCl-induced unfolding, allows reconstruction of the first structural changes after dissociation of the native dimer and illustrates the mechanism by which Pfk-2 unfolding occurs.

Single-molecule studies of adenylate kinase protein under force.

Wilson, C[1].,Leachman, S[2].,Marqusee, S[3].,Bustamante, C[3].,[1]Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y farmacéuticas, Universidad de Chile.[2]Department of Chemistry, QB3, University of California at Berkeley.[3]Quantitative Biosciences, QB3, University of California at Berkeley.

Single-molecule manipulation has increasingly become a useful method for studying macromolecular dynamic. In this study we used the optical tweezers (OT) and magnetic tweezers combined with fluorescence to study the mechanical stability of adenylate kinase (AK) from the thermophilic organism *Aquifex*.This protein is a monomer and has three domains. AK was first characterized in OT and was found to unfold around 25 pN during force-extension experiments with a fast (mseg) 4 nm intermediate at 15 pN. This intermediate could correspond to the ATP binding domain unfolding independently of the rest of the protein. We have built an instrument for force spectroscopy that combines the capabilities of magnetic tweezers and single molecule Förster resonance energy transfer. A magnet exerts force on 2.1 µm antidigoxigenin-coated paramagnetic beads tethered to AK. These fluorescently labeled proteins are functionalized with DNA handles containing biotin and immobilized on the surface of a flow chamber in a total internal reflection fluorescent microscope via streptavidin interactions. The distance between dyes from each molecule that is attached to a bead in the microscope's field of view can be monitored as a function of force. This enzyme was successfully labeled with fluorescent dyes and DNA using click chemistry and cysteine chemistry. Preliminary fluorescence data from our instrument confirms the existence of this intermediate under force. FONDECYT 11130263, CB-HHMI, SM-NSF.

Type II topoisomerases, structure, mechanism and drug inhibition.

Laponogov I[2].,Veselkov, D[2].,Umrekar, T[2]., Isabelle M.-T., C[1].,Pan, Xiao-Su[1].,Selvarajah, Jogetha[1].,Fisher, L[1].,**Sanderson, M[2].**, [1]Division of Biomedical Sciences, St. Georges, University of London, Cranmer Terrace, London SW17 0RE, U.K..[2]3rd Floor New Hunts House, Division of Medical and Life Sciences, Division of Cell and Molecular Biophysics, Kings College, Guys Campus, London Bridge, London SE1 1UL, U.K..

Type II topoisomerases perform essential roles in DNA replication, chromosome segregation, and recombination and are very important antibacterial and anticancer targets. Type II topoisomerases regulate DNA supercoiling and chromosome segregation via an ATP-driven DNA strand passage mechanism. However, the paucity of structures for native full-length proteins has been a significant obstacle in defining the reaction pathway. Here will be presented a high resolution X-ray crystal structure of an 'open clamp' complex of a type II topoisomerase, the key complex engaged in DNA capture and transport. The topoisomerase IV structure shows the disposition and conformation of all three gates required for catalysis and reveals a novel DNA binding site providing new insight on DNA bending and distortion at the pivot about flexible hinges to capture the incoming DNA. The open clamp state is the starting point for the topo IV reaction cycle, and its structure allows us to draw the overall mechanistic pathway by which coordinated minimal movement of domains results in DNA strand passage. Recent topoisomerase II-DNA-drug complex structural studies will also be discussed.

Microfluidics in Quantitative Biology Symposium

Self-organization in bacterial colonies

Federici, F[1]., Rudge, Tim[2].,[1]Genetica Molecular y Microbiologia Universidad Catolica de Chile.[2]Plant Sciences, Research associate, University of Cambridge.

We use bacterial colonies as multicellular models for the engineering of artificial patterning and morphogenesis. We have developed tools for measuring, modelling and engineering mechanical and genetic processes in bacteria. This talk addresses two interlinked projects:

1. The study of physical self-organization in bacterial biofilms

We have used fast, large-scale biophysical and genetic modeling tools for simulating biofilms. Using these tools and confocal microscopy we have revealed emergent properties of cell-shape that give rise to self-similar fractal-like morphology in biofilms, and confirmed this with high resolution confocal microscopy. I will present these results, and show how this approach can inform engineering of multicellular systems.

2. The use of novel Synthetic Biology methods for the characterization of artificial genetic modules at different conditions of growth.

We are developing relative (or ratiometric) characterization of genetic components and modules; to inform high-throughput computational modeling used to evaluate attainable regions of network space. We are using this approach to build artificial gene regulatory networks that will be useful in engineering complex multicellular behavior.

Microfluidic platforms for high-throughput single-cell analysis: studies in size control mechanisms and nucleoid organization

Nugent, E[1]., [1]Physics Department, Cavendish Laboratory, Biological and Soft Systems, University of Cambridge.

In this talk I will present two examples which demonstrate the power of microfluidic approaches in generating large data sets for quantitative investigations of microbiological questions. (i) How do cells cell growth and division to effect size control? Focusing on E. coli in steady growth, we quantify cell-division control using a stochastic model, by inferring the division rate as a function of the observable parameters from large empirical data sets of dividing cells (ii) What is the role of the nucleoid in effecting the global transcriptional changes which drive physiological adaptation in response to environmental perturbations? We use a microfluidic device to maintain bacterial populations in a well-defined physiological state before applying a rapid nutrient perturbation and characterizing the response of supercoiling sensitive promoters.

Hidden behind the population mean: single cell analysis of membrane recruitment dynamics of the mating MAPK pathway scaffold protein.

Colman, A[1]., [1]Fisiología, Biología Molecular y Neurociencias Universidad de Buenos Aires.

In *S. cerevisiae*, pheromone activates a GPRC coupled to a MAPK cascade pathway that, among other effects, arrests cell cycle progression in G1. However, in cells committed to a new round of cell division, Cdk activity blocks pheromone response. Plasma membrane (PM) recruitment of the mating MAPK scaffold, Ste5, is a key step in pheromone signaling. It is this membrane interaction that is inhibited by Cdk activity by phosphorylating residues flanking the Ste5 PM binding domain. Recently we developed a quantitative method to measure protein relocalization over time using microscope cytometry. Here, using this method, we studied the early dynamics of Ste5 recruitment as a function of the cell cycle position in single live cells. In contrast to our expectations, we found that initial recruitment is similar in G1 and S phase cells, but then it rapidly declines in S phase cells. Remarkably, this decline in S phase is strictly dependent on the activity of the mating MAPK Fus3. Nevertheless, Fus3 activity alone cannot displace Ste5 from the membrane, since Ste5 recruitment persists over time in G1 cells or when it lacks the Cdk sites flanking its PM domain. These findings reveal that Fus3, on top of its classic mating promoting functions, cooperates with the Cdk in a complex negative feedback, bringing about distinct patterns of temporal dynamics at different cell cycle stages.

Biophysics of cell assemblages in microfluidic-based synthetic ecosystems

Keymer, J[1]., [1]Ecologia Pontificia Universidad Católica De Chile.

Nano fabricated structures can be used to study cellular biophysics of bacteria in the context of landscape evolutionary ecology and in a spatially distributed synthetic ecosystem. I will tell two stories about how we in the lab have been using these on-chip “cellular ecologies” to study the complexity of life at two fundamental levels of organization: (i) from the cells down to molecular networks which make cells, and (ii) cell assemblages where, many cells make ecological communities or/and eventually tissues.

Bioinformatics Brazil-Chile Symposium

Metagenomics of composting: bioinformatics and results.

Setubal, J[1]., [1]Bioquimica, Instituto de Quimica, Universidade de Sao Paulo.

Ithis talk I will present an overview of the metazoo project, which investigates the microbial diversity in composting samples obtained in the Sao Paulo Zoo, in Brazil. I will describe the various computational tools that we are developing and/or using to analyze time series next-generation sequencing datasets. I will aso present preliminary results, based on more than 100 million reads from more than 30 samples.

Computational Engineering of Immunoreactive HIV-1 Antigens

Lins, R[1]., [1]Fundamental Chemistry Federal University of Pernambuco.

Protein scaffolding is one of the main strategies for epitope exposure. However conformational instability of the engineered chimeras often leads to loss of native conformation followed by degradation and/or aggregation, therefore limiting success rate. To overcome this issue, a novel computational protocol combining de novo and molecular dynamics techniques has been devised to address protein scaffolding in a predictive manner. Such approach is showcased by the design of immunoreactive gp41-based conformation-specific HIV-1 epitopes grafted onto highly-stable scaffolds aimed to point-of-care diagnostic kits and vaccines. The computer-engineered recombinant proteins were produced in bacteria using codon optimized DNA sequences and their diagnostic performance was assessed by microarray and surface plasmon resonance against a cohort of over 100 patient sera samples.

Molecular Dynamics Simulations to understand the structure-function properties of potassium channels

Gonzalez, W[1]., [1]Center for Bioinformatics and Molecular Simulations University of Talca. (Sponsored by Fondecyt 1140624)

Molecular dynamics simulations (MDS) capture the behavior of biological macromolecules in full atomic detail. Using homology modeling and MDS we have been studied since 2006 the structure-function properties of potassium (K^+) channels, which catalyze rapid, selective, and regulated K^+ fluxes across membranes. Within this talk, two gating mechanism of two different K^+ channels will be analyzed by means of homology modeling, MDS and functional assays. In the mammalian K_2P channel TASK-3 the cooperative gating by extracellular pH will be explained and the voltage-sensor transitions of the voltage-gated K^+ channel KAT1 of *Arabidopsis thaliana* will be shown. By the end of the talk the relentless growth in computational power, the development of new algorithms and the improvement of force-field parameters for MDS will be discussed. All the previous aspects will absolutely benefit the study of the structure-function properties of K^+ channels, increasing the timescales accessible to MDS by several orders of magnitude

Assesment of methods for comparing networks of gene coexpression

González, L[1].,Medina, F[1].,**Verdugo, R[1].**, [1]Programa de Genética Humana, Facultad de Medicina, Universidad de Chile.

Graphs are commonly used to represent gene networks. However, how to best assess similarity between networks using graphs remains an open question. Nine distance metrics from the literature and two from this work were compared. Using simulated data, we tested which metric maximized recall and precision to detect a desired network among a set 50 random networks. The Maximum Common Subgraph Distance (mcsd) gave the best results, even when random changes were applied to the target network. Thus, mcsd was chosen as the best metric to identify similar networks among datasets. We then estimated empirical null distributions for each metric using a resampling approach with a real dataset. Gene expression data from the San Antonio Family Heart Study was used (1,100 Mexican-Americans, 65% women, 15-65 years old, 18,525 genes expressed in lymphocytes). K-means clustering was performed and repeated after data were split in two random samples. The network distance between similar clusters in either split was recorded and the process was repeated 1000 times. Large differences in distributions among clusters were observed for all three metrics, only partially explained by cluster size. We conclude that significance of network differences can be evaluated by resampling but in a cluster-by-cluster basis.

Development of improved methods to calculate ligand-binding affinities

Ryde, U[1]., Söderhjelm, P[1].,Genheden, S[1].,Mikulskis, P[1].,Olsson, M[1].,Andrejic, M[2].,Mata, R[2].,[1]Department of Theoretical Chemistry, P. O. Box 124, SE-221 00 Lund, Sweden, Lund University.[2]Department of Theoretical Chemistry Lund University.

The prediction of the binding free energy of a drug candidate to its target receptor by theoretical methods is one of the most important challenges to computational chemists. A large number of methods have been suggested, ranging from strict physical methods like free-energy perturbations (FEP) and thermodynamic integration (TI) to statistical scoring functions. Intermediate in this range are end-point methods, i.e. methods based on simulations of only the bound and unbound states of the ligand. Examples of the latter methods are the MM/GBSA (molecular mechanics combined by generalised Born solvation and surface area calculations) and LIE (linear interaction energy) methods. During recent years, we have tested and tried to improve many of these approaches:

- We have show how the precision of the MM/PBSA entropy term can be improved.
- We have developed a strategy to obtain MM/GBSA results with a statistical precision of <1 kJ/mol and have shown how the sampling can be improved by independent trajectories.
- We have developed improved methods to obtain atomic charges[4] and we have tested the use of different values of the dielectric constant.
- We have tested the influence of different polar and non-polar solvation terms.
- We have compared the results and efficiency of MM/GBSA with LIE, FEP, and TI.
- We have developed methods to improve the efficiency of FEP.
- We have developed methods to use various QM methods (semiempirical to LCCSD(T)) to calculate binding affinities.
- We have performed a large test of FEP methods for >100 ligands
- We have tested the approximations involved in MD simulations and MM/GBSA.

In this talk, I will describe some of these results.

Cell Signaling and Disease Symposium

Cardiolipin is a Key Determinant for mtDNA Stability and Segregation

Kowaltowski, A[1]., [1]Departamento de Bioquímica Universidade de São Paulo. Luis Alberto Luévano-Martínez, Maria Fernanda Forni, Valquiria Tiago dos Santos, Nadja C. Souza-Pinto, Alicia J. Kowaltowski

Mitochondria play a key role in adaptation during stressing situations. Cardiolipin, the main anionic phospholipid in mitochondrial membranes, is expected to be a determinant in this adaptive mechanism since it modulates the activity of most membrane proteins. Here, we used *Saccharomyces cerevisiae* subjected to conditions that affect mitochondrial metabolism as a model to determine the possible role of cardiolipin in stress adaptation.Interestingly, we found that thermal stress promotes a 30% increase in the cardiolipin content and modifies the physical state of mitochondrial membranes. These changes have effects on mtDNA stability, adapting cells to thermal stress. Conversely, this effect is cardiolipin-dependent since a cardiolipin synthase-null mutant strain is unable to adapt to thermal stress as observed by the 60% increase in cells lacking mtDNA (r^0). Interestingly, we found that the loss of cardiolipin specifically affects the segregation of mtDNA to daughter cells, leading to a respiratory deficient phenotype after replication. We also provide evidence that this segregation defect is linked to a close physical association between cardiolipin and mtDNA. Overall, our results demonstrate that the mitochondrial lipid cardiolipin is a key determinant in the maintenance of mtDNA stability and segregation.

Funding: FAPESP, CNPq, Redoxoma.

New insights into IGF-1 signaling in the heart

Lavandero, S[1]., [1]Advanced Center for Chronic Diseases (ACCDiS), Facultad Ciencias Quimicas y Farmaceuticas/ Facultad Medicina, Universidad De Chile. (Sponsored by FONDAP 15130011 (SL), ANILLO ACT 1111 (SL))

Insulin-like growth factor 1 (IGF-1) signaling regulates contractility, metabolism, hypertrophy, autophagy, senescence, and apoptosis in the heart. IGF-1 deficiency is associated with an increased risk of cardiovascular disease, whereas cardiac activation of IGF-1 receptor (IGF-1R) protects from the detrimental effects of a high-fat diet and myocardial infarction. IGF-1R activates multiple pathways through its intrinsic tyrosine kinase activity and through coupling to heterotrimeric G protein. These pathways involve classic second messengers phohosphorylation cascades, lipid signaling, Ca^{2+} transients, and gene expression. In addition, IGF-1R triggers signaling in different subcellular locations including the plasma membrane, perinuclear T tubules, and also in internalized vesicles. In this presentation, I will provide a fresh and updated view of the complex IGF-1 scenario in the heart, including a critical focus on therapeutic strategies.

Plasma exosomes from rats and humans protect the myocardium from ischemia-reperfusion injury

Davidson, S[1]., [1]The Hatter Cardiovascular Institute University College London.

Ischaemic heart disease is the leading cause of death globally. Myocardial infarction causes mortality and morbidity in patients with coronary artery disease. Reperfusion is necessary to salvage the myocardium but causes further cell death called “reperfusion injury”. It is therefore necessary to develop approaches to minimize reperfusion injury. Exosomes are nanometer-sized circulating vesicles, which mediate inter-cellular communication by ferrying diverse proteins and nucleic acids. Exosomes are present at high quantities in the blood of healthy individuals, and their effects are virtually unknown. Here we studied plasma exosomes as possible cardioprotective agents.

Exosomes were isolated from the plasma of rats and healthy human volunteers. Administration of these exosomes to isolated, Langendorff-perfused hearts protected them from ischaemia and reperfusion injury (vehicle: 35±3%; Exosomes: 21±3%Infarct/AAR; $p<0.01$). Furthermore, i.v. administration of exosomes also reduced infarct size in an *in vivo* rat model (vehicle: 48±7%; Exosomes: 21±4%Infarct/AAR; $p<0.01$). Exosomes directly protected primary cardiomyocytes when added before hypoxia-reoxygenation *in vitro* (43±7% reduction in cell death, $p<0.01$). The mechanism of cardioprotection was found to involve signaling via Toll-like Receptor 4 (TLR4) which activated the MEK/ERK/p38MAPK kinase signaling pathway and resulted in phosphorylation of the small heat shock protein, Hsp27.

Exosomes released from HUVEC endothelial cells were also able to deliver cardioprotective stimuli, suggesting that, *in vivo,* local signaling via exosomes may modulate resistance of the heart to ischemia-reperfusion injury. Plasma exosomes appear to mediate cardioprotection via an evolutionarily ancient pathway encompassing the innate immune system and HSPs.

Dissecting microRNA mechanisms of action to better understand their roles in disease.

Ricci, E[1]., [1]RNA Therapeutics University of Massachusetts Medical School.

microRNAs (miRNAs) are small non-coding RNAs that regulate gene expression in many cellular processes. miRNAs act as guides for the RNA-induced silencing complex (RISC) to bind messenger RNAs (mRNAs), repress their translation and induce their decay. Supporting their importance in gene regulation, over 60% of mammalian mRNAs contain conserved miRNA binding sites in their 3’ untranslated regions. Furthermore, disregulation of miRNA expression or activity has been linked to many human diseases including cancer.

Deciphering the molecular pathways leading to miRNA-mediated gene regulation have uncovered a complex and multi-step mechanism involving various translation and RNA decay factors. Among them, recent reports including those from our laboratory, point to the translation initiation factor eIF4A as a critical target of microRNA activity. Furthermore, our results revealed that not all mRNAs are repressed to the same extend upon miRNA binding. Interestingly, eIF4A is required for translation of many oncogenes and has been recently shown to contribute to cancer progression. Here, we will discuss the molecular mechanism of miRNA-mediated gene regulation and link those findings to their role and impact in disease states.

New Members Sessions

New Members Session 1

Genomic gains and losses in triple negative breast tumors, in relation with BRCA1 expression and localization.

Tapia, T[1]., Aravena, A[2].,Alvarez, C[1].,Cornejo, V[3].,Fernández, W[3].,Camus, M[4].,Maass, A[2,5].,Carvallo, P[1].,[1]Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile.[2]Centro de Modelamiento Matemático Universidad De Chile.[3]Unidad de Anatomía Patológica Hospital San Borja Arriarán.[4]Centro de Cáncer, Facultad de Medicina, Pontificia Universidad Católica de Chile.[5]Departamento de Ingeniería Universidad de Chile. (FONDECYT 1080595, CONICYT 24091058.)

Triple-negative breast cancer, defined by the absence of expression of estrogen and progesterone receptors and HER2, represent a group of tumors not having directed therapy. Therefore, knowing the genes involved in this type of cancer is highly relevant for treatment. We studied 48 triple-negative breast tumors by immunohistochemistry for cytokeratins CK5, CK14, and CK8/18, EGFR and BRCA1. Using array-CGH, we characterized common gains and losses between tumors. Expression and localization of BRCA1 analysis revealed that 20.8%(10/48) of tumors presented normal nuclear expression, 31.3%(15/48) has absent/low nuclear expression and 47.9%(23/48) has altered BRCA1 localization in the cytoplasm. These results suggest that the loss of expression of BRCA1 or its altered localization may be relevant for tumor progression. Array-CGH analysis revealed regions of gains (5q15.33, 20q13.33, 21q22.3) and losses (1q21.1, 6p11.2; 11q22.3) shared by more than 60% of these tumors. Unsupervised hierarchical clustering of these tumors separated two main groups, one of which correlates with the low expression/miss-localization of BRCA1. In addition, specific copy number alterations were found in each group according to BRCA1expression/localization. Analysis of the genes contained in these regions revealed specific biologicals processes and signaling pathways affected in tumors with normal expression of BRCA1 and absent/low expression BRCA1.

Bipolar Plasma Membrane Distribution of Phosphoinositides and Their Requirement for Auxin-Mediated Cell Polarity and Patterning in *Arabidopsis*.

Tejos, R[1,2]., Sauer, M[1,2].,Vanneste, S[1,2].,Palacios-Gomez, M[3].,Li, H[3].,Heilmann, M[4].,Van Wijk, R[5].,Vermeer , J[5].,Heilmann, I[4].,Munnik, T[5].,Friml, J[1,2,3].,[1]Department of Plant Systems Biology VIB, Belgium.[2]Department of Plant Biotechnology and Bioinformatics Ghent University, Belgium.[3]Institute of Science and Technology (IST), Austria.[4]Department of Cellular Biochemistry Martin-Luther-University Halle-Wittenberg, Germany.[5]Swammerdam Institute for Life Sciences, Section Plant Physiology University of Amsterdam, Netherlands.

Cell polarity manifested by asymmetric distribution of cargoes, such as receptors and transporters, within the plasma membrane (PM) is crucial for essential functions in multicellular organisms. In plants, cell polarity (re)establishment is intimately linked to patterning processes. Despite the importance of cell polarity, its underlying mechanisms are still largely unknown, including the definition and distinctiveness of the polar domains within the PM. Here, we show in *Arabidopsis thaliana* that the signaling membrane components, the phosphoinositides phosphatidylinositol 4-phosphate (PtdIns4P) and phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2] as well as PtdIns4P 5-kinases mediating their interconversion, are specifically enriched at apical and basal polar plasma membrane domains. The PtdIns4P 5-kinases PIP5K1 and PIP5K2 are redundantly required for polar localization of specifically apical and basal cargoes, such as PIN-FORMED transporters for the plant hormone auxin. As a consequence of the polarity defects, instructive auxin gradients as well as embryonic and postembryonic patterning are severely compromised. Furthermore, auxin itself regulates PIP5K transcription and PtdIns4P and PtdIns(4,5)P2 levels, in particular their association with polar PM domains. Our results provide insight into the polar domain–delineating mechanisms in plant cells that depend on apical and basal distribution of membrane lipids and are essential for embryonic and postembryonic patterning.

The crystal structure of Ferritin from *Chlorobium tepidum* reveals a new conformation of the 4-fold channel for this protein family.

Yévenes, A[1]., Arenas-Salinas, M[2].,Towsend, P[3].,Matias, C[4].,Watt, R [4].,Brito, C[5].,Marquez, V[5].,Marabolli, V[5].,Gonzalez-Nilo, F[5].,López-Castro, J[6].,Dominguez-Vera, J[6].,Ehmke, P[3].,[1]Química Física, Facultad de Química, Pontificia Universidad Católica De Chile.[2]Centro Bioinformática y Simulación Molecular, Facultad de Ingenería, Universidad De Talca.[3]Department of Chemistry and School of Biological and Biomedical Sciences Durham University.[4]Department of Chemistry and Biochemistry Brigham Young University.[5]Centro Bioinformática y Biología Integrativa, Facultad de Ciencias Biológicas, Universidad Andrés Bello.[6]Departamento de Química Inorgánica Universidad de Granada. (This Work Was Supported By Conicyt: Proyecto Anillo ACT 1107 And A Santander Mobility Grant From Durham University. We Would Like To Thank Ian Edwards For His Technical Support In Crystallization, And The Diamond Beam Line Staff For Providing And Maintain)

Ferritins share a common architecture made of 24 subunits of five a-helices. The recombinant *Chlorobium tepidum* ferritin (rCtFtn) is a structurally interesting protein since sequence alignments with other ferritins show that this protein has a significantly extended C-terminus, which possesses 12 histidine residues as well as several aspartate and glutamic acid residues that are potential metal ion binding residues. We show that the macromolecular assembly of rCtFtn exhibits a cage-like hollow shell consisting of 24 monomers that are related by 4-3-2 symmetry; similar to the assembly of other ferritins. In all ferritins of known structure the short fifth a-helix adopts an acute angle with respect to the four-helix bundle. However, the crystal structure of the rCtFtn presented here shows that this helix adopts a new conformation defining a new assembly of the 4-fold channel of rCtFtn. This conformation allows the arrangement of the C-terminal region into the inner cavity of the protein shell. Furthermore, two Fe(III) ions were found in each ferroxidase center of rCtFtn, with an average FeA-FeB distance of 3 Å; corresponding to a diferric μ-oxo/hydroxo species. This is the first ferritin crystal structure with an isolated di-iron center in an iron-storage ferritin. The crystal structure of rCtFtn and the biochemical results presented here, suggests that rCtFtn presents similar biochemical properties reported for other members of this protein family albeit with distinct structural plasticity.

New Members Session 2

Using Phylogenomics to Characterize Significantly Mutated Protein Superfamilies from Tumor Exomes

Almonacid, D[1]., Pizarro, D[1].,Soto, J[1].,Bascur, J[1].,Varas, I[1].,[1]Center for Bioinformatics and Integrative Biology, Facultad de Ciencias Biológicas, Universidad Andrés Bello. (Supported By: Grant Fondecyt 11130578, Chile, To DEA.)

Mutations identified in genome sequences of tumors are normally interpreted at the gene level, missing important biological context that homologous genes can provide for understanding the molecular and biological basis of cancer. We try to solve this problem by characterizing cancer mutations in proteins considering the sequence-structure-function paradigms of the superfamilies to which they belong using protein similarity networks, a new phylogenomics approach. In these networks, nodes represent protein sequences, and edges correspond to sequence similarities among nodes. The objective of the technique is to identify the best clade in which to interpret the mutation data for a protein in the context of its homologs. Whole-exome somatic mutations for 4327 patients affected by one of 19 different types of cancer were obtained from The Cancer Genome Atlas. Significantly mutated proteins for each type of cancer were identified using MutSigCV. We generated protein similarity networks for significantly mutated proteins in these data, and then extended the networks to include other eukaryotic proteins, and then proteins from all domains of life. We studied the topologies of the resulting networks to evaluate whether information from other organisms improved or not the clades identified. We also evaluated the use of clustering and thersholding of edge values in the networks for better discrimination of clades. So far, we have characterized the impact of cancer mutations in metabolic enzymes, DNA binding proteins, and proteins known to aggregate.

Folding pathways, conformational stability and mechanical properties of proteins with knotted topologies.

Bustamante, A., Reyes, J., Wilson C. A. M[5] .,Guerra, D[1].,Bustamante, C[2,3,4].,**Baez, M[5]**., [1]Lima, Perú, Universidad Peruana Cayetano Heredia.[2]Berkeley, CA, USA, QB3 California Institute for Quantitative Biosciences.[3]Berkeley, CA, USA, Jason L.Choy Laboratory of Single-Molecule Biophysics.[4]Berkeley, CA, USA., Howard Hughes Medical Institute.[5]Bioquímica y Biología Molecular, Ciencias Químicas y Farmaceuticas, Universidad De Chile.

Knots are natural topologies created by thermal fluctuation of artificial and natural polymers like DNA. However, polypeptide chains of some proteins have the ability to self-knot reliably upon reaching their native or stable 3D structure. Although these types of structures are scarce, is not clear how their folding mechanism occurs or if there is any functional significance associated with the knotted topology of the polypeptide chain. In our lab we have compared the folding mechanism of proteins with or without knots of members of the superfamily of RHH transcription factors. Using optical tweezers, both types of proteins were stretched upon pulling their C and N-terminal extremes. Results show elevated mechanical resistance against forces in the case of the protein with a knot. Further analysis and comparison of the force-extension data from both type of proteins allowed to us determining the thermodynamic consequence of a knot on the conformational stability of the polypeptide chain. Additionally, to determine which terminus threads the polypeptide chain, a knotted protein was fused to a hyperstable protein to create a steric impediment. The C-terminus fusion avoided the folding of a knot while the N-terminus fusion did not affect its folding. Considering these results, knotted topologies impact the stability and mechanical resistance of proteins and induce a polarized pathway of folding.
Fondecyt 11110534.

Discovery of selective 11β-HSD1 inhibitors by combined ligand- and structure-based virtual screening

Lagos, C F[1]., Vecchiola, A[1]., Allende, F[2]., Solari, S[2].,Baudrand, R[1].,Campino, C[1].,Cifuentes, M[3].,Owen, G[4].,Carvajal, C[1].,-Fardella, C[1].,[1]Department of Endocrinology, School of Medicine, Pontificia Universidad Católica De Chile.[2]Department of Clinical Laboratories, School of Medicine, Pontificia Universidad Católica De Chile.[3]Institute of Nutrition and Food Technology (INTA) Universidad De Chile.[4]Department of Physiology, Faculty of Biological Science, Pontificia Universidad Católica De Chile. (Supported By FONDEF CA12i10150, CORFO 13CTI-21526-P1, SOCHED 2013-6, IMII P09/016-F, & FONDECYT 1130427 Grants. OpenEye, ChemAxon & Inte:Ligand For Academic Licenses.)

11 beta-hydroxysteroid dehydrogenase type 1 (11β-HSD1) converts cortisone to cortisol in a NADPH dependent manner. Overexpression of 11β-HSD1 in key metabolic tissues is related to the development of type 2 diabetes, obesity, dyslipidemia, hypertension and metabolic syndrome. Using all the available crystal structures of human 11β-HSD1 in complex with inhibitors as source of structural information, a combined ligand and structure-based virtual screening approach was implemented to identify novel 11β-HSD1 inhibitors. A selected group of compounds was identified in silico and evaluated in cell-based assays for cytotoxicity and 11β-HSD1 mediated cortisol production inhibitory capacity. The expression of 11β-HSD1 and 11β-HSD2 in human LS14 adipocytes was assessed during differentiation. Biological evaluation of the series of compounds in adipocytes and steroid determination by HPLC-MS/MS identify a set of compounds which selectively inhibit 11β-HSD1 mediated cortisol production (reductase activity) with potencies in the micromolar range, and showed to be selective against the isoform 11β-HSD2. The identified scaffolds represents novel leads that might serve as starting point for the development of more potent derivatives with higher efficacies targeting intracellular cortisol levels in type 2 diabetes and metabolic syndrome.

Identification of genetic and epigenetic biomarkers of response to lipid-lowering drugs

Salazar, L[1]., [1]Ciencias Básicas, Medicina, Universidad de La Frontera.

Variability in drug efficacy and drug safety is a major challenge in current clinical practice, drug development, and drug regulation. Sequence variations in drug target proteins, enzymes, and transporters can alter both, drug efficacy and side effects, to cause variable responses in individual patients. The focus of our main research line, over the last 10 years, has been to evaluate the influence of gene polymorphisms involved in pharmacokinetics and pharmacodynamics of different lipid-lowering drugs. Our data showed that polymorphisms in *CYP3A4*, *ABCG5*, *PCSK9*, *APOE*, *SREBP-2*, *SLCO1B1* and *LDLR* genes explain, partly, the individual variability to statins, drugs commonly used to lower cholesterol levels by inhibiting the HMG-CoA reductase enzyme, in Chilean subjects. However, these polymorphisms cannot fully explain variability in response to these drugs, promoting the need to generate different approaches. Due to the absence of studies based on deregulation of epigenetic mechanisms in subjects undergoing statin therapy, research in this field can provide important information that may help clarify mechanisms and molecular pathways involved in statins response variability. Recent data of our laboratory shows that statins deregulate microRNA expression signature *in vitro* and *in vivo*. Six microRNAs were downregulated (miR-29a-3p, miR-29b-3p, miR-300, miR-33a-5p, miR-33b-5p and miR-454-3p) in peripheral mononuclear cells from patients with hypercholesterolemia after 1 month treatment with atorvastatin (10 mg/day). Furthermore, our data showed that statins induce DNA hypomethylation and modifications in H3 and H4 histones *in vitro*. Financial support: FONDECYT (1130675), FAPESP (2011/21967-1) & CNPq (473485/2012-5)

New Members Session 3

Mitochondrial metabolism and the control of vascular smooth muscle phenotype

Chiong, M[1]., Morales, PE[1].,Torres, G[1].,Cartes-Saavedra, B[1].,Norambuena-Soto, I[1].,Mondaca-Ruff, D[1].,Vidal-Peña, G[1].,Nuñez-Soto, C[1].,García-Miguel, M[1].,Michea, L[2].,[1]ACCDiS, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad De Chile.[2]ICBM, Facultad de Medicina, Universidad De Chile. (Sponsored by Fondecyt 1110180, Fondecyt 1140329, FONDAP 15130011, Anillo ACT1111.)

Differentiation and dedifferentiation of vascular smooth muscle cells (VSMCs) are essential processes of vascular development. VSMC have biosynthetic, proliferative and contractile roles in the vessel wall. Alterations in the differentiated state of the VSMC play a critical role in the pathogenesis of a variety of cardiovascular diseases, including atherosclerosis, hypertension and vascular stenosis. We propose that cell metabolism, in particular mitochondrial function and autophagy, are important regulators in the phenotypic change of VSMC. Mitochondrial activity can be controlled by regulating mitochondrial dynamics, i.e. mitochondrial fusion and fission, and by regulating mitochondrial calcium content through the interaction with the endoplasmic reticulum (ER). GLP-1-dependent activation of mitochondrial fusion and mitochondria-ER interaction can prevent VSMC dedifferentiation as detected by VSMC proliferation and migration assays. Moreover, PDGF-BB, Ang II and TNF-β induce VSMC dedifferentiation and autophagy. Inhibition of autophagy completely prevents VSMC phenotypic change. Therefore, our data suggest that cell metabolism is a key factor in the control of VSMC phenotype, indicating a new area to be explored in the treatment of vascular diseases.

Role of the morphogenetic factor, CdeC, in the assembly of the exosporium layer of *Clostridium difficile* spores.

Milano, M[1].,Olguín, V[1].,**Paredes-Sabja, D[1].**, [1]Departamento de Ciencias Biológicas, Facultad de Ciencias Biológicas, Universidad Andrés Bello. (Sponsored by Fondecyt Regular 1110569)

Clostridium dificile is an important nosocomial pathogen that has become a major cause of antibiotic-associated diarrhea. *C. difficile* spores are essential for *C. dificile* pathogenesis, infection and persistence of the disease. The exosporium is the outermost layer of *C. difficile* spores plays roles in early host-spore interactions such as spore adherence to intestinal epithelium cells. We have identified a cysteine rich exosporium morphogenetic factor required for correct exosporium assembly. In this study, by using translational fusions of exosporium proteins, we first demonstrate that the exosporium proteins BclA1, BclA2, BclA3, CdeA and CdeB and the exosporium morphogenetic factors CdeC and CdeM are uniquely localized in the exosporium layer of *C. difficile* spores. Strikingly, nearly 20 and 30% of total levels of previously described spore surface proteins CotA and CotB, respectively, were localized in the exosporium layer and the majority was coat-located. CdeC is involved in formation of high molecular mass complex (> 120 kDa) of BclA1, BclA2 and BclA3. In absence of CdeC, BclA proteins are present as 40 kDa species. CdeC also plays a role exosporium localization of CotB, but not CotA or the morphogenetic factor CdeM. Collectively, this work provides a first approach of the mechanisms associated in the assembly of the exosporium layer of *C. difficile* spores.

Fondecyt Regular 1110569

Protective role of the ACE2/Ang-(1–9) axis in hypertension and cardiovascular damage

Ocaranza, M[1]., Moya, J[1].,Escudero, N[1].,Barrientos , V[2].,Novoa , U[3].,Michea, L[3].,Chiong, M[4].,Jalil, J[1].,Lavandero , S[4,5].,[1]Cardiovascular Diseases Division, Faculty of Medicine, Pontifical Catholic University of Chile.[2]Integrative Phisiology Laboratory, Faculty of Medicine , University of Chile.[3]Biomedical Sciences Department, Faculty of Sciences , University of Talca .[4]Advanced Center for Chronic Diseases (ACCDiS), Faculty of Chemical and Pharmaceutical Sciences, University of Chile .[5]Department of Internal Medicine, Faculty of Medicine, University of Texas Southwestern Medical Center. (Sponsored by FONDEF D11I1122 (MPO; SL; MC), FONDAP 15130011 (SL; MC).)

Cardiovascular (CV) diseases remains the most prevalent cause of human morbidity and mortality. The Chronic renin-angiotensin system (RAS) activation by angiotensin (Ang) II leads to CV diseases and perpetuates a cascade of prohypertrophic, proinflammatory, prothrombotic, and atherogenic effects associated with CV damage. In 2000, a new pathway consisting of angiotensin-converting enzyme 2 (ACE2), Ang-(1-9), Ang-(1-7), and the Mas receptor was discovered. Activation of this novel pathway stimulates vasodilation, antihypertrophy, and antihyperplasia. For some time, studies focused mainly on Ang-(1-7), and theMas receptor, and their biological properties that counterbalance the ACE/Ang II axis. No previous information about Ang(19) suggested that this peptide had biological properties. However, our data suggest that Ang-(1-9) protects the heart and blood vessels (and possibly the kidney) from adverse CV remodeling in patients with hypertension (HT) and/or heart failure. These beneficial effects are not modified by the Mas (Ang-(1-7) receptor) blocker, but they are abolished by the Ang II type 2 receptor (AT2R) antagonist. Current information suggests that the beneficial effects of Ang-(1-9) are mediated via the AT2R. In conclusion Ang-(1-9) is a new vasoactive peptide of the RAS that prevents and decreases HT as well as pathologic CV and kidney damage and dysfunction.

Coupling nuclear export and translation of the HIV full-length unspliced mRNA

Soto-Rifo, Ricardo[1]., [1]Programa de Virología, ICBM, Facultad de Medicina, Universidad De Chile. (Sponsored by Fondecyt 11121339)

Human Immunodeficiency virus (HIV) gene expression is highly complex and tightly regulated. Viral transcription depends on the host RNAPII and involves the synthesis of different transcripts that are classified depending on their splicing status as fully spliced, partially spliced and full-length unspliced. As a classical cellular mRNA, viral transcripts that are fully spliced follow the canonical mRNA metabolism pathway including the splicing-dependent recruitment of multisubunit complexes such as the TREX complex and the exon-junction complex, which are responsible of coupling NXF1-dependent nuclear export with translation. In sharp contrast, gene expression from partially spliced and the unspliced viral transcript requires the use of an alternative nuclear export pathway in order to avoid quality control mechanisms that normally retain and degrade misprocessed, intron-containing, mRNAs within the cell nucleus. As such, the viral protein Rev binds exclusively to this class of viral transcripts and drives their nuclear export through the non-canonical CRM1-dependent pathway. Our research is focused on the post-transcriptional control on HIV, trying to understand the mechanisms by which the virus exploits host factors to accomplish efficient gene expression. By using the full-length unspliced mRNA as a model, we have gained insights into the alternative strategies employed by the virus to couple nuclear export and translation. We also evaluate these processes as potential targets for pharmaceutical intervention.

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Oral Sessions

Oral Session 1 Gene expression and Inmunology

Impact of the cellular context on the IRES activity of the human T-cell lymphotropic virus type 1 (HTLV-1) and the human immunodeficiency virus type 1 (HIV-1)

Olivares, E[1].,Cáceres, C[1].,Pino, K[1].,López-Lastra, M[1].,[1]Laboratorio de Virología Molecular, Escuela de medicina, Pontificia Universidad Católica De Chile. (Sponsored by FONDECYT 1130270 Y P09/016-F Iniciativa Científica Milenio Del Ministerio De Economía, Fomento Y Turismo. Eduardo Olivares Was Supported By UCH0604 MECESUP-USACH Doctoral Fellowships.)

The full length mRNA of HTLV-1 and HIV-1 harbors an IRES element. Studies used to identify this elements mostly relay on the transfection of bicistronic reporter plasmids. The use of this system has been strongly criticized.It is recognized that a caveat to the bicistronic reporter approach is false-positive IRES activity attributable to cryptic promoter activity or splicing of the tested sequence within cells. In this study we sought to further characterize translation initiation from a bicistronic reporter plasmid that harbor the IRES elements of HIV-1 and HTLV-1 in the context of different cell lines including Cos-7, HeLa, HEK293T,Vero, Vero E6, NHI-3T3 and Jurkat cells. The results show the HIV-1 and HTLV-1IRES activity is dependent of the cellular context. Additionally, we observed that the translational efficiency is higher in Cos-7, HEK293T and Jurkat cells. This suggests that both IRESs are functional in cells that correspond to the natural targets of infection, T-lymphocytes. Additionally, we show that in some cellular types, the putative IRES activity can be in fact explained by the presence of a cryptic promoter. This study establishes that the activity of the cryptic promoter present within the used bicistronic mRNAs is cell type dependent.

Use of nano particles of chitosan to deliver oral vaccines in aquaculture

Rivas-Aravena, A[1]., Fuentes, Y[1].,Cartagena, J[1].,Sandino, A[1].,Spencer, E[1].,[1]Biología, Química y Biología, Universidad De Santiago De Chile. (This Work Was Supported By Fondecyt De Postdoctorado 3120149 And Conicyt ACE-02. The Authors Wish To Thank Dr. Michel Brémont For Kindly Donating The Alphaviral Vector)

Salmoniculture in Chile and other countries is currently threatened by different pathogens that are not been efficiently treated by developed vaccines. This fact could be explained because i) vaccines are mainly administered by intraperitoneal injection, provoking stress to the fish and injury to the filet, and ii) the adjuvants used in aquaculture could provoke side effect to the fish. In this investigation we developed an oral vaccine against ISAV encapsulated in nanoparticles of chitosan. These nanocapsules are mainly spherical having a diameter of 100 nm. Nanoparticles are able to deliver their cargo *in vitro* into a fish cell line and *in vivo* to Atlantic salmon. The oral administration of the vaccine in the food to Atlantic salmons caused a fast stimulation of the innate immune response. Results obtained in the present investigation suggest that nanoparticles of chitosan are a powerful strategy that could be effective to develop a new generation of vaccines in aquaculture.

TRIM protein expression in RT-gill cell line challenged with LPS and Poly I:C

Donoso, F[1]., Álvarez, C[1].,Santana, P[1].,Mercado, L[1].,[1]Laboratorio de Genética e Inmunología Molecular, Facultad de Ciencias, Pontificia Universidad Católica De Valparaíso.

The immune system in teleost fish must display alert systems for defense against pathogen attack at epithelial lining tissues. The gills are a mucosal tissue in permanent contact with the environment which makes it vulnerable to the entry of infectious organisms. As a highly irrigated tissue, immune activity is strongly influenced by circulating peripheral leukocytes. Thus, the potential use of gill tissue as an indicator of health risk in farmed fish requires the characterization of immune response markers expressed in this tissue. TRIM proteins (Tripartite motif proteins) are ancestral molecules in biological systems which act as intracellular cytokines and are associated with transcriptional regulation of immune regulatory genes. In order to identify the ability of intracellular immune response in the gill tissue of the rainbow trout, RT-gill cell line were challenged with LPS and Poly I:C. Results showed the induction of TRIM transcripts by both challenges using conventional PCR. Amplified products were further purified and cloned for sequencing. The primary structure analysis of the encoded protein reveals molecules displaying either two or three of the three motifs that define the TRIM proteins. The relative expression of TRIM proteins in response to challenge will be measured by qPCR to establish the magnitude of the response. These results support the potential use of TRIM proteins in gill tissue as indicators of infection in response to both LPS and Poly I:C challenge.

Construction and validation of TAL effectors as tool for enhancing the activity of IL-8 promoter region from *Oncorhynchus mykiss*

Sequeida, A[1]., Cardenas, C[2].,Marshall, S[1].,Mercado, L[1].,[1]Laboratorio de Genética e Inmunología Molecular, Ciencias, Pontificia Universidad Católica De Valparaíso.[2]NBC, NBC, Pontificia Universidad Católica De Valparaíso.

TAL (Transcription activator-like) effectors are useful tools for genome engineering, due to its high specificity to DNA sequences that can be used for different purposes such as enhancing gene expression and induction of specific mutations. In this work we constructed and validated four TAL effectors specific to four different zones of Interleukin 8 (IL-8) promoter region from rainbow trout to enhance its activity. The IL-8 promoter region (Acc. No: FM206384) was cloned into pGL3 (Promega) vector to obtain pIL8PR vector. Effectors were constructed using Golden Gate TALEN and TAL effector kit 2.0. As destination vectors, we use pTALE64 with VP64 transcription activator to obtains vectors T1 to T4. To validate the promoter activity induction, COS-7 cell line were co-transfected using vectors pIL8PR with T1 to T4. After 48 hours post transfection, cells were lysed using Bright-Glo (Promega) reagent and after 2 minutes, Relative Light Units (RLU) was measured and data normalized against total protein content. Results show that every treatment has statistically significant differences compared to control condition. Treatment with T3 alone was the most effective, showing more than 257-fold promoter activity induction, followed by treatments using effector pairs T2/T3, T1/T2 and T2/T4, but there was no statistically significant difference between this last three treatments. These results validate the use of TAL effectors for further experiments using different rainbow trout cell lines.

Genes from plasmids of tellurite-resistant bacteria confer resistance to tellurite and other metal(loid)s in *Escherichia coli*.

Cornejo, F[1]., Muñoz-Villagrán, C[1].,Figueroa, M[1].,Arenas, F[1].,Vásquez, C[1].,[1]Biología, Química y Biología, Universidad De Santiago De Chile. (Sponsored by FONDECYT Regular 1130362 And INACH DG_03-13)

Some metal(loid)s are essential for life because they can catalyze (or to participate in) reactions through their particular chemical properties. Conversely, others are extremely toxic even at nanomolar concentrations, like tellurite. Despite this toxicity, there exist some organisms that are able to resist the toxicant effects. This is due in part to the existence of genetic resistance determinants encoded in the genome or in plasmids. We recently isolated bacterial strains from antarctic territory that tolerate high tellurite concentrations (~500 fold more than *E. coli*). Some of them carry plasmids that were purified and sequenced. Five of these extrachromosomal elements were sequenced using Ion Torrent technologies and then ORF\'s were searched using bioinformatic approaches. Subsequently primers were designed to amplify each ORF which were cloned into pBAD expression vector and transformed into *E. coli* LMG 194. Plasmids encode proteins that seem to be involved in resistance to cold (CspA), metal (CzcD, ATP-dependent translocase of Cu) and antibiotics (aminoglycoside acetyltransferase). Cells carrying these cloned genes were analyzed through MIC, growth inhibition zones and growth curves. Preliminary results show that some of these ORF's, when expressed in *Escherichia coli*, increase the cell's resistance to tellurite and other metals.

Regulation of the ascorbic acid transporter SVCT1 during the differentiation of human intestinal CaCo-2 cells

Guzmán, P., Muñoz, A[1].,Villagrán, M[1].,Barra, M[1].,Vera, J[1].,Rivas, C[1].,[1]Fisiopatología, Facultad de Ciencias Biológicas, Universidad De Concepción.

The differentiation of intestinal epithelial cells includes changes in the expression of genes involved in nutrient uptake. We show here that the differentiation of human intestinal CaCo-2 cells is accompanied by increases in ascorbic acid transport and SVCT1 expression at the protein and mRNA levels, and that the increased SVCT1 expression is transcriptionally regulated. To better understand the regulatory aspects involved, we performed a structural-functional analysis of the proximal promoter of the *SVCT1* human gene. We cloned a 1.5 kb segment containing the proximal promoter, generated partial constructs of decreasing sizes by deletion with restriction enzymes and mutant promoters by site-directed mutagenesis, and analyzed their capacity to direct the transcription of a reporter gene after transfection in human CaCo-2 cells, including co-expression of transcription factors whose consensus binding sequences are present in the promoter. This analysis revealed the presence of two regulatory sites, for HNF1 and HNF4, crucial for the transcriptional activity of the human *SVCT1* gene promoter. Overexpression of these transcription factors in the CaCo-2 cells indicated that HNF1 activated while HNF4 repressed the transcriptional activity of the SVCT1 promoter. Thus, the promoter region of SVCT1 responds to transcription factors that play a central role in the regulated expression of genes associated with differentiation of intestinal cells.

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Translation regulation of the HBZ protein in the human T-cell lymphotropic virus type 1 (HTLV-1)

Cáceres, J[2,1]., Olivares, E[2,1].,Castillo, E[2,3].,Pino, K[2].,Lopez-Lastra, M[2].,[1]Programa Doctorado Microbiología Universidad de Chile/Universidad de Santiago.[2]Laboratorio de Virologia Molecular, Escuela de Medicina, Pontificia Universidad Católica De Chile.[3]Programa Doctorado en Genetica molecular y Microbiología Pontificia Universidad Católica De Chile.
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HTLV-1 synthesizes a pool of antisense messenger RNAs (mRNAs) from the provirus 3'LTR. Two different mRNAs had been reported that codes for two distinct version of the HBZ protein. One of these is generated by a spliced mRNA (spHBZ) and the other by a unspliced mRNA (usHBZ). Both mRNAs posses different 5' untranslated regions (5'UTR). The spHBZ version is expressed most in T-cells which suggest a posible translation regulation. In this work, monocistronic vectors were generated wich contains the 5'UTR of spHBZ or usHBZ mRNAs fused to the sequence of the firefly luciferase gene. The results shows that the spHBZ mRNAs have more translational activity than the usHBZ mRNA in ex vivo experiments suggesting a translational regulation in the HBZ protein synthesis. In HeLa cells it was determinated that the effect observed can't be explained by a transciptional effect. RNAs transfections experiments show similar result, wich is consistent with the idea that spHBZ and usHBZ proteins are regulate in a translational level. In Rabbit reticulosite lysate system, we demostrate that the translation regulation depend of the 5' Cap structure eliminating the possibility that the higher expression of the HBZ protein generated by spHBZ mRNA is due to the presence of a IRES element. C.J. Cáceres and E.S. Olivares should be considered as first authors.

Mercuric reductase (MerA) confers tellurite resistance in bacteria

Muñoz-Díaz, P[1]., Rodríguez, F[1].,Díaz-Vásquez, W[1].,Arenas, F[1].,Vásquez, C[1].,[1]Biología, Quimica y Biologia, Universidad De Santiago De Chile. (Sponsored by Funding:FONDECYT 1130362 And Dicyt USACH)

The tellurium oxyanion tellurite (TeO_3^{-2}) is highly toxic for most bacteria. Toxicant detoxification occurs mainly by reduction to a less toxic form (Te^0) at the expense of cellular thiols like glutathione or through flavoprotein enzymes that display tellurite reducing activity. These include the enzyme mercuric reductase (MerA), encoded in the *mer* operon, which is responsible of bacterial Hg^{2+} resistance by catalyzing the reduction of Hg^{2+} to the less toxic, elemental form Hg^0. With that said, we hypothesized that MerA could reduce tellurite, hence promoting both tellurite as well as mercury resistance. To evaluate this hypothesis we isolated mercury-resistant bacteria from the Antarctic territory and determined if cross-resistance (mercury/tellurite) occurs in these isolates. The results showed that strains containing the *mer* operon are significantly more resistant to tellurite in the presence of mercury, suggesting that mechanisms involved in mercury resistance (eg. the *mer* operon) could induce tellurite resistance. Moreover, purified MerA from one of the isolates can efficiently reduce tellurite *in vitro* and *E. coli* expressing this recombinant enzyme exhibits an enhanced tellurite resistance phenotype.

Oral Session 2 Molecular Cell Biology I

Molecular and functional analysis of genes involved in the synthesis and accumulation of flavonoids during fruit development in Vitis Vinifera L. CV. CARMÉNÈRE

Pérez-Díaz, R[1]., Pérez-Díaz, J[1].,González, E[1].,Ruiz-Lara, S[1].,[1]Instituto de Ciencias Biológicas Universidad de Talca. (Sponsored by FONDEF G07I1003)

In grapevine, anthocyanins and proanthocyanidins (PAs) are the main flavonoids present in fruits, which are associated with red wine organoleptic properties. Flavonoid pathway is specifically regulated at transcriptional level and several R2R3-MYB proteins have shown to act as positive regulators. Here, we present the characterization of *VvMYB4-like* gene, a new R2R3-MYB repressor factor in grapevine, which in highly expressed in skin berry at pre veraison stages. Heterologue expression in tobacco resulted in the loss of pigmentation in flowers due a decrease in anthocyanin accumulation associated to the down-regulation of flavonoid-related genes. Once flavonoids are synthesized in the cytoplasm, they are transported to the vacuole or other compartments for storage. Three types of proteins have been associated to this process: glutathione S-transferase, MATE-type transporters and a proton pump encoded by *AHA10* in *Arabidopsis*. In this regard, homologue genes in grapevine were investigated. *VvAHA10.1* was expressed primarily in fruit skins and its overexpression in tobacco increased the flower pigmentation. *VvMATE1* and *VvMATE2* were highly homologues to PA transporters in plants and their expression profile correlated with PAs accumulation in grapevine. MATE1 and MATE2 proteins showed different subcellular localization, suggesting that both proteins could mediate the transports and accumulation of PAs through different routes and cellular compartments.

Genome-wide transcript profiling to uncover new genes in the SCF-TIR1/AFBs-independent pathway of lateral root formation in *A. thaliana.*

Pérez-Henríquez, P[2]., Parizot, B[1].,Chen, Q[1].,Beeckman, T[1].,Norambuena, L[2].,[1]Department of Plant Systems Biology VIB, Gent, Belgium.[2]Centro de Biología Molecular Vegetal, Facultad de Ciencias, Universidad de Chile.

Root systems are crucial for plant fitness. Root system architecture relies largely on the continuous process of lateral root initiation (LRI). It is well known that the hormone auxin is key in LRI. We have previously described Sortin2 as a unique molecule that induces LRI independently of the auxin receptor SCF-TIR1/AFBs. This work attempts to reveal the main biological processes targeted specifically by Sortin2. It also aims at selecting plausible candidate genes for further characterization of its molecular pathway. To understand the specific Sortin2 effect in LRI we performed and compared a genome-wide transcript profiling of Sortin2- and auxin-treated seedlings roots. Analysis with a 2-fold change threshold showed that Sortin2 treatment modify transcript levels of a 3 times smaller group of genes than auxin, suggesting a narrower effect of Sortin2. Substracting auxin- from Sortin2-modified genes, yielded a small subset of 171 and 182 genes that were specifically up- and down-regulated in Sortin2 treatments, respectively. Response to stimulus either endogenous, external or biotic and signal transduction processes were enriched among Sortin2- specific up-regulated genes, suggesting a unique molecular frame for Sortin2-induced LRI. Among Sortin2 enriched processes we found highly regulated transcription factors that might be setting the transcriptional program needed for a SCF-TIR1/AFBs-independent LRI pathway. FONDECYT1120289, PhD FELLOWSHIP & GO21110627 CONICYT, DPP UCHILE

Novel extra-circadian functions of the FREQUENCY protein in B. cinerea: implications in conidiation and sexual development.

Müller, H[1]., Hevia, M[1].,Canessa, P[1].,Larrondo, L[1].,[1]Genética Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica De Chile. (Sponsored by FONDECYT 1131030, MN-FISB NC120043)

Botrytis cinerea is considered the second most important fungal phytopathogen, due to the economical relevance and wide variety of its hosts. Although circadian clocks are conserved throughout the tree of life, due to the adaptive advantage that they confer to their carriers, they have not been studied in this fungus. We found in *B. cinerea* the three major components of a circadian oscillator, corresponding to the homologues of *Neurospora crassa* White Collar 1 (WC-1), White Collar 2 (WC-2) and Frequency (FRQ). FRQ inhibits its own expression by blocking the transcriptional activity of the White Collar Complex, formed by WC-1 and WC-2, closing a transcriptional-translational feedback loop (typical of a circadian oscillator). BcFRQ1 oscillates at the transcriptional and translational levels under constant conditions, and is essential for the functioning of this clock. When analyzing knock out strains of *bcfrq1*, we found important developmental problems, leading to the production of sexual structures such as microconidia and sclerotia. These phenotypes are intriguing, since they don't seem to be related to clock effects, suggesting extra-circadian functions for BcFRQ1. In order to explore these new BcFRQ1 roles, and their effect on pathogenicity, we are examining defects in signaling pathways, as well as analyzing RNAseq data of a *bcfrq1* mutant strain.

The endocytosis dynamics inducer Sortin2 triggers founder cell specification involving a distinctive lateral root formation transcriptional program in *Arabidopsis thaliana*

Morales, S[1]., Pérez-Henríquez, P[1].,Norambuena, L[1].,[1]Centro Biología Molecular Vegetal, Facultad de Ciencias, Universidad De Chile. (Sponsored by FONDECYT 1120289)

Plants are exceptionally plastic modulating their development postembryonically in response to changes in environmental conditions. Roots constitute a fundamental organ for plant physiology, being able to modulate its architecture developing lateral roots (LR). Root pericycle cells are primed to be founder cells that follow a strictly organized cell division program to originate a LR primordium. Recently, by means of the synthetic compound Sortin2, we have published that endocytosis dynamics induction promotes LR formation (LRF) by a mechanism independent of canonical auxin receptor. The goal of ourwork is understanding the mode of action of Sortin2 over the LRF program and how this is executed. We have found that Sortin2 promotes *de novo* LRF suggesting its effect over cell specification of founder cells. Consistently Sortin2 is able to induce organogenesis locally indicating that promotes early steps of LRF. Indeed mutants with defects on LR initiation are resistant to Sortin2 placing its effect upstream of their molecular gene function. Interestingly, genes that have GO term *LR development* are predominantly transcriptionally regulated by auxin, however, these genes are just slightly modulated in Sortin2 treatments. Overall, our results strongly suggest the existence of a distinctive LR triggering program stimulated by endocytosis dynamics induction that promotes founder cell specification conducive to LRF for root architecture remodeling in *A. thaliana*.

FoxO1 switches energy metabolism and stimulates mitochondrial fission in cardiac myocytes

Quiroga, C[1]., Acuña, F[1].,Vásquez-Trincado, C[1].,Riveros, C[1].,Lavandero, S[1,2].,[1]Advanced Center for Chronic Diseases (ACCDiS) and Center for Molecular Studies of the Cell (CEMC), Faculty of Chemical and Pharmaceutical Sciences & Faculty of Medicine, University of Chile.[2]Department of Internal Medicine UT Southwestern Medical Center.

The cardiomyocytes are highly differentiated cells and responsible of myocardial contraction. Their large requirements of energy make them particularly sensitive to changes in intracellular energy metabolism. Recently, we showed that cardiomyocyte mitochondrial morphology and metabolism are regulated by insulin. On the other hand, the transcriptional factor FoxO1 is negatively regulated by insulin but its activation also down-regulates insulin signaling pathways (i.e. insulin resistance and type 2 diabetes mellitus). We evaluate here the role of FoxO1 on cardiomyocyte mitochondrial morphology and energy metabolism. To this end, FoxO1-GFP or the constitutive active form (FoxO1CA-GFP, MOI 25) were expressed or silenced with siRNA for FoxO1 in cultured rat cardiomyocytes. Our data showed that expression of FoxO1CA stimulated a fragmented mitochondrial phenotype with changes in the expression of mitochondrial dynamics related genes (Drp-1, Mfn-2 and Opa-1). In parallel, FoxO1CA-GFP decreases oxidative metabolism protein expression and mitochondrial metabolism, assessed by qPCR, mitochondrial membrane potential, oxygen consumption, ATP synthesis and ROS production. FoxO1CA also reduces GLUT4 transcription and ^{3}H-2D-glucose uptake. We concluded that FoxO1 stimulates a metabolic switch and mitochondrial fission in cardiac myocytes.

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Mitochondrial transport of vitamin C

Roa, F[1]., Muñoz, C[1].,González, M[1].,Sotomayor, K[1].,Peña, E[1].,Inostroza, E[1].,Salvatori, O[1].,Vera, J[1].,Rivas, C[1].,[1]Fisiopatología, Ciencias Biológicas, Universidad De Concepción.

Ascorbic acid (reduced vitamin C) transporters have been described at the plasma membrane level, but little is known at subcellular level. Although the importance of mitochondria in the redox cell metabolism has been widely established, there is no clarity regarding the mechanism of transport of vitamin C in this organelle. We report here that human HEK-293 cells express a mitochondrial low-affinity ascorbic acid transporter that molecularly corresponds to SVCT2. Confocal colocalization experiments with anti-SVCT2 and anti-organelle protein markers revealed that most of the SVCT2 immunoreactivity was associated with mitochondria, with minor colocalization at the endoplasmic reticulum. Immunoblotting of proteins from highly purified mitochondrial fractions confirmed that SVCT2 was associated with mitochondria, and transport in isolated mitochondria revealed a sigmoidal ascorbic acid concentration-response curve with an apparent ascorbic acid transport K_m of 0,6 mM. SVCT2-siRNA decreased mitochondrial SVCT2 protein expression by approximately 75%, with a concomitant decrease in the mitochondrial ascorbic acid transport rate. These results indicate that SVCT2 is localized in mitochondria in HEK-293 cells and is responsible for the uptake of ascorbic acid in this organelle. We propose that the mitochondrial localization of SVCT2 is a property shared across different cells, tissues and species.

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fraroa@udec.cl

Primary cilia as mechanosensors in the heart

Villalobos, E[1]., Hill, J[2].,Criollo, A[3].,G, Diaz-Araya[4].,Lavandero, S[5].,[1]Departamento de Bioquimica y Biologia Molecular, Facultad de Ciencias Quimicas y Farmaceuticas, Universidad De Chile.[2]Department Internal Medicine, Medical Center, University of Texas Southwestern .[3]Instituto de investigacion en Ciencias Odontologicas, Facultad de Odontologia, Universidad De Chile.[4]Advanced Center for Chronic Diseases (ACCDiS) & Center for Molecular Studies of the Cell (CEMC), Faculty of Chemical and Pharmaceutical Sciences, Universidad De Chile.[5]Advanced Center for Chronic Diseases (ACCDiS) & Center for Molecular Studies of the Cell (CEMC), Faculty of Chemical and Pharmaceutical Sciences & Faculty of Medicine, Universidad De Chile.

Cardiovascular diseases are the leading cause of mortality worldwide. In response to stress or acute ischemia the heart develops a strong pathological setting, which offsets cardiac wall stress pointing towards heart failure and fibrosis. The primary cilium is a protuberant membrane structure, which, in response to changes in pressure or fluid flow, can bend activating intracellular pathways depending on cell type, tissue and stimulus. In this context, we propose “primary cilium” is a key player in the mechanotransduction of signals in the heart. Even though studies show that most of the cells express the cilium, up to now, it is unknown whether cilia are localized in the heart tissue. Our aims were: to evaluate if the cilium is present in the cardiac tissue, to study in which type of cells is expressed in the heart & to determine its role in the control of cardiac fibrosis. To this end, neonatal cardiomyocytes and cardiac fibroblasts were isolated from rat. The presence of cilia and PC 1 and PC2 proteins were evaluated by Immunofluorescence assay. Myocardial infarction was produced in C57/BL6 mice by ligation of the left anterior descending artery. Two weeks later, hearts were harvested and fixed. Our results showed that cilia were identified in epicardial cells in embryonic hearts. Further, cilia were enriched in hearts infarcted areas. IHC studies revealed that ciliated cells were in cardiac fibroblasts. We concluded that cilia are found in epicardial cells in hearts from embryos. In neonatal and adult mice, cilia are localized in cardiac fibroblasts.

Regulation of AGO2 function and degradation during glutathione depletion

Mancilla, H[1]., Slebe, J[1].,Meister, G[2].,Concha, I[1].,[1]Instituto de Bioquímica y Microbiología Universidad Austral de Chile.[2]Laboratory for RNA Biology University of Regensburg. (Sponsored by FONDECYT 1110508 (IC) Y 1141033 (JCS). HM: Becario Doctorado CONICYT, DID-UACH 1330-32-06 Y Beca Estadía MECESUP AUS 1203)

miRNAs interact with Argonaute (Ago) proteins to form RNA-induced silencing complex (RISC) and guide them to specific target sites located in the 3´-UTR of target mRNAs leading to translational repression and deadenylation-induced mRNA degradation. Autophagy is the major intracellular degradation system by which cytoplasmic proteins and organelles are delivered and degraded in the lysosome. Ago2 is directed for degradation as miRNA-free entities by the selective autophagy receptor NDP52. We have previously described that a glutathione (GSH) deficiency triggers autophagy. The aim of this work was to evaluate the degradation of Ago2 during a glutathione depletion condition. Here, we show that Hela cells treated with L-buthionine-(S,R)-sulfoximine (BSO), a potent inhibitor of glutathione biosynthesis, decreases the protein level of Ago2. However, when Hela cells were treated with BSO and chloroquine to inhibit autophagy, accumulation of Ago2 was observed. We found that Ago2 localization and its binding site to the 5´-end of the miRNA are important for its degradation during this condition. Finally GSH depletion and autophagy inhibition affect the interaction of Ago2 with Let7a miRNA. These results suggest that GSH and autophagy machinery are important for the normal gene silencing through miRNAs.

Oral Session 3 Protein Structure and Computational Biology

Structural-functional analysis of the oligomeric structure of the human ascorbic acid transporter-2 (SVCT2).

Gatica, M[1]., Sweet, K[1].,Muñoz, A[1].,Aylwin, C[1].,Reyes, A[2].,Rivas, C[1].,Vera, J[1].,[1]Fisiopatología, Ciencias Biológicas, Universidad De Concepción.[2]Instituto de Bioquímica y microbiología, Ciencias, Universidad Austral De Chile. (Sponsored by Juan Carlos Vera Cárcamo)

SVCT2 is a sodium-coupled ascorbic acid transporter with an apparent ascorbic acid transport K_m of approximately 20 µM, from which we have limited structural information. We analyzed SVCT2 quaternary structure and its effect on ascorbic acid transport. Cross-linking with PFA, DSS and BS3 followed by western blot showed formation of protein complexes with apparent molecular masses consistent with dimerization. Coexpression of mutant and native proteins within a cell is a very powerful method to study potential interactions between polypeptides. We coexpressed in HEK-293 cells increasing amounts of SVCT2, simultaneously with decreasing amounts of a low-affinity SVCT2 mutant (SVCT2-m, a conformational mutant with an apparent ascorbic acid transport K_m > 100 µM). A single kinetic component was observed at all ratios of SVCT2/SVCT2-m, without evidence of two kinetic components. Interestingly, the apparent transport K_m in cells coexpressing SVCT2 + SVCT2-m was similar to that of the transporter that is expressed in greater proportion; ≈ 30 µM in excess of SVCT2 and ≈ 150 µM in excess of SVCT2-m. These results suggest that SVCT2 is present in vivo as a functional oligomer of two subunits, that the minimum transport unit within the dimer would be the monomer, and that the monomers in the dimer are able to interact with each other modulating their kinetic properties.

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marcgatica@udec.cl

Isolation and characterization of the major hemolymph protein from *Choromytilus chorus*

Hernandez, M[1]., Artigues, A[2].,Villar, M[2].,Vanacore, R[3].,Concha, M[4].,Amthauer, R[4].,[1]AUSTRAL-omics, Ciencias, Universidad Austral De Chile.[2]Biochemistry and Molecular Biology Department, Medical Center, Kansas University.[3]Vanderbilt Medical Center, Nephrology Faculty, Vanderbilt University.[4]Instituto de Bioquimica y Microbiologia, Facultad de Ciencias, Universidad Austral De Chile. (Sponsored by AUSTRAL-omics MECESUP-AUS0807)

The main hemolymph protein of the majority of bivalves corresponds to a high molecular weight protein whose biological function has not been established yet. We have isolated this protein (PMHC) from *Choromytilus chorus* hemolymph and demonstrated that it corresponds to an acidic histidine-rich glycoprotein (pI=5.9) displaying a homomultimeric quaternary structure with a particle of 40nm under native conditions, and with a monomer size of 75 kDa. The amino terminal sequence of PMHC, obtained by Edman degradation, matches perfectly with the sequence of the main hemolymph protein from *Perna canaliculus* (pernin). The analysis of the tryptic map using the Sequest tool allowed confirming that PMHC corresponds to a pernin-like protein. Also, PMHC *de novo* sequencing was performed using mass spectrometry, obtaining the sequence of several peptides that align with pernin, cavortin and dominin, all corresponding to major proteins from bivalve hemolymph. *In silico analyses* show that all four proteins share the sequence ACCV and also a common superoxide dismutase (SOD) domain which is triplicated in pernin. It is interesting to highlight that PMHC displays antimicrobial activity against *E. coli in vitro*, suggesting that this protein could be a component of the innate immune system of *C. chorus*. In addition, this protein constitutes a very good antigen since a high titer antiserum was obtained in rabbits immunized only with the purified protein. Based on its abundance, easy purification, size and antigenicity it has biotechnological potential as a carrier protein.

Computational study of COR (cold-regulated) proteins of *Arabidopsis thaliana* during cellular dehydration.

Navarro, C[1]., Alzate-Morales, J[1].,Caballero, J[1].,González, W[1].,Hincha, D[2].,[1]Centro de Bioinformática y Simulación Molecular, Facultad de Ingeniería, Universidad de Talca.[2]Molecular Plant Physiology Max-Planck-Institute. (Sponsored by C.N.R. Thanks A Doctoral Fellowship Awarded By Government Of Chile Through CONICYT No 21120691.)

Cold has a major influence on plant growth and survival. Considerable effort has been directed towards understanding how the model plant *Arabidopsis thaliana* adapts to low temperature. In response to cold the *C-repeat binding factors (CBF)* of *A. thaliana* are rapidly induced and in turn activate the transcription of a set of target genes including the COR/LEA (Late Embryogenesis Abundant) protein-encoding genes.

COR/LEA proteins play a crucial role by improving cell resistance during cellular dehydration. COR/LEA proteins are intrinsically disordered proteins on water, but can acquire secondary structure during cellular dehydration. Based on Circular Dichroism (CD) spectroscopy analysis it has been seen that the proteins COR15A (At2g42540) and COR15B (At2g42530) of *Arabidopsis thaliana* on solvent are mostly unstructured, but after dehydration the unstructured content decreased significantly, acquiring secondary structure (mostly α-helix). Even more, Fourier transform infrared spectroscopy analysis has shown that these two proteins can stabilize liposomes membranes on *in vitro* analysis during partial and complete dehydration.

On that way we aim to explain the structural changes of both COR15 proteins during cellular dehydration by performing *all-atom* molecular dynamics simulations on glycerol-water mixtures solvents in order to represent the peculiar behavior of these protein in response of water loose.

Structural model of a respiratory syncytial virus (RSV) matrix protein dimer

Schüller, A[2,1]., Ríos-Vera, C[2].,Gutiérrez, F[2].,Melo, F[2].,[1]Molecular Bioinformatics Laboratory Millennium Institute on Immunology and Immunotherapy.[2]Depto. Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile. (Acknowledgements: FONDECYT No. 1131065 And ICM P09-016-F)

Human respiratory syncytial virus (hRSV) is an enveloped RNA virus and is the principal cause of bronchiolitis and pneumonia in infants worldwide. The viral M protein plays a central role in virus assembly and budding; and M oligomerization is discussed to be critical for production of infectious hRSV. Despite evidence for dimeric and higher order forms in solution, hRSV-M was crystallized as a monomer. Here we present a structural model of a potential dimeric quaternary structure of hRSV-M. We performed a systematic analysis of mononegavirus matrix proteins and identified 22 related crystal structures. Several related matrix proteins were crystallized in a particular planar square-shaped, dimeric or tetrameric quaternary structure, and served as templates for comparative modeling. Dimeric hRSV-M models were generated by a multi-template approach with help of the software MODELLER and were validated by a statistical potential derived from known protein complexes. Surface features of dimeric hRSV-M were in good agreement with experimental results for hRSV-M RNA binding, and agreed with the current model for membrane association. In addition, residues participating in the dimer interface were evolutionary conserved. In absence of a dimeric crystal structure of hRSV-M, our results might help to improve the understanding of oligomerization and interaction with viral and host factors on an atomic level.

Molecular determinants of the functional properties of the ascorbic acid transporter-2 (SVCT2).

Sweet, K[1]., Gatica, M[1].,Muñoz, A[1].,Escobar , M[1].,Peña, E[1].,Aylwin, C[1].,Salas- Burgos , A[1].,Reyes, A[2].,Rivas , C[1].,Vera, J[1].,- [1]Fisiopatología, Ciencias Biológicas , Universidad De Concepción.[2]Instituto de Bioquímica y Microbiología , Ciencias , Universidad Austral De Chile. (Sponsored by Juan Carlos Vera)

We used the crystal coordinates of the bacterial UraA transporter to construct a folding 3D model of the human ascorbic acid transporter SVTC2. The SVCT2 3D model contains 14 transmembrane segments (TMS) spatially organized into a core (TMS 1–4 and TMS 8–11) and a gate domain (TMS 5-7 and TMS 12-14). We used the 3D model of SVCT2 refined by molecular dynamics (5 ns) to perform molecular docking studies with the substrate L-ascorbic acid to identify amino acid residues possibly involved in substrate binding and translocation along the transport channel. Each amino acid residue was replaced with alanine using site-directed mutagenesis, and the resulting mutant proteins were expressed in HEK-293 cells and analyzed for protein expression by immunoblot, cellular localization by confocal microscopy, and functional properties by ascorbic acid transport assays. All mutant proteins were efficiently expressed and were present at the plasma membrane, and two sets of amino acid residues were identified through the functional analysis, a group of residues located in the TMS of the core domain whose substitution altered the transport K_m, and a second group located outside the core domain whose substitution affected the activation by sodium. We conclude that the ascorbic acid and sodium binding sites in SVCT2 can be functionally uncoupled without simultaneously affecting the ascorbic acid transport Km and the sodium cooperativity (nH).

Conicyt Fellowship, Fondecyt grants 1130842 and 1140429.
kasweet@udec.cl

Emergence of pyridoxal phosphorylation through a promiscuous ancestor during the evolution of hydroxymethyl pyrimidine kinases.

Castro-Fernandez, V[1]., Bravo-Moraga, Felipe[1].,Ramirez-Sarmiento, Cesar[1].,Guixé, Victoria[1].,[1]Departamento de Biología, Facultad de Ciencias, Universidad De Chile. (Sponsored by Fondecyt 1110137)

In the family of ATP-dependent vitamin kinases from the ribokinase superfamily, we found enzymes that phosphorylate hydroxymethyl pirimidine (HMPK) and other enzymes that phosphorylate pyridoxal (PLKs). Interestingly, several bifunctional enzymes related to the HMP kinases have been described. To determine how bi-functionality emerged in HMP Kinases, we reconstructed the sequence of three ancestors of these enzymes, resurrected them experimentally and assayed the enzymatic activity of the last common ancestor (ancC). The resurrected ancestral enzyme showed a Km of 28 mM for pyridoxal and 7 mM for HMP. Also, ancC has 8-fold higher specificity for HMP compared to pyridoxal phosphorylation, which prompted us to consider this activity as a promiscuous one, since the high Km value for PL would not be physiologically relevant. This preference for HMP as substrate is related to the presence of a glutamine residue (Gln44), which is proposed to be a key determinant of the specificity towards this substrate. The promiscuous activity of enzymes has been proposed as the starting point for new activities during evolution. In our case, this trait would have allowed the appearance of an activity already present in this family (PLK), in a convergent and independent manner.

New computational strategies to understand the conductance mechanism in K+ channels.

Gonzalez, F[1,3]., Sepúlveda, Romina[1,2].,Bravo, Felipe[1].,Latapiat, Veronica[1].,Diaz-Franulic, I[3,4].,Naranjo, David[3].,[1]Center for Bioinformatics and Integrative Biology (CBIB), Facultad de Ciencias Biologicas , Universidad Andres Bello.[2]Programa de Doctorado en Biotecnología Universidad Andrés Bello.[3]Centro Interdisciplinario de Neurociencias de Valparaíso Universidad De Valparaíso.[4]Programa de Doctorado en Ciencias m/Neurociencias Universidad De Valparaíso. (This Work Was Supported By FONDECYT 1131003 (FGN) And CINV (Millenium Initiative, 09-022-F), RS Thanks To CONICYT For Doctoral Scholarship)

The mechanism underlays the conductance and gating of potassium channels has been highly studied using electrophysiological, structural and computational approaches. However, one key question has not been elucidated yet: Despite the fact that the structure of the SF is conserved among K+ channels, why do they show dissimilar conductance rates? e.g. 250 pS for BK channel and 20 pS for Shaker channel.

In order to answer this question, we were able to implement two computational approaches: 1) Application of an external electric field into a Molecular Dynamics Simulation and 2) Generation of a double bilayer system in order to represent the differences of potential.

We have observed the ion translocation process in a high and low conductance K+ channels using an external electric field application. Unexpectedly, the permeation rate seems related to the potassium dehydration and the pore size, due to the water molecules distribution is different in each pore. Also, we observed that the configuration of ions into the selectivity filter change between Shaker and BK channel.

This study provides new perspectives to understand the ion conductance observed in high and low conductance K+ channels, allowing to propose new hypotheses which were validated through site directed mutagenesis and electrophysiological assays.

Homology and Pharmacophore Modeling, High Throughput Virtual Screening and Molecular Docking Studies to Identify Potential Inhibitors of the Two-pore-Domain Potassium Channel K_{2P}9.1 (TASK-3).

Ramirez, D[1]., González, W[1].,Zuñiga, L[2].,Arevalo, B[1].,[1]Centro de Bioinformática y Simulación Molecular, Facultad de Ingeniería, Universidad de Talca.[2]Escuela de Medicina, Escuela de Medicina, Universidad de Talca. (Sponsored by Fondecyt 1140624, Fondef CA13I10223)

Two-pore domain potassium channels (K_2P) give rise to leak potassium currents, which control the excitability of the cells. The human genome contains 15 KCNK genes coding for proteins able to form K_2P channels subdivided into 6 subfamilies on the basis of their sequence similarities. Members of TASK subfamily such as TASK-3 channel are inhibited by extracellular acidification. TASK-3 channel is principally abundant in the hippocampus, cerebellum and cortex. The development of new selective TASK-3 inhibitors could influence the pharmacological treatment of several neurological conditions.

In this study, an e-pharmacophore model from several TASK-3 blockers was developed based on 5,6,7,8-tetrahydropyrido-[4,3-d]pyridine analogues with a IC_{50} range of 1 – 0.035 µM. The e-pharmacophore hypothesis was tested against the ZINC database. Four hits were found through High Throughput Virtual Screening and molecular docking. The lead ligands are ZINC59268134, ZINC09703892 and ZINC29084017 (ZINC database), and the compound schrod975000 (Schrödinger drug-like data set). The lead ligands docked on TASK-3 channel, were subject to 10 ns molecular dynamics simulations (MDs) to verify the stability of the complex through the time.

We consider that the characterization of TASK channels through the study of their interactions with different molecules could help to improve the rational design of new drugs targeting these proteins and to generate new therapeutic treatments relating to pH-gated K_2P channels diseases.

Oral Session 4 Biomedicine and Gene Expression

Primary cultures of advanced cervical cancer as model for selective antisense therapy

Ávila, R[1]., Farfán, N[1].,Villota, C[1,2].,Dadlani, K[1].,Lobos-Gonzales, L[1].,Socias, M[3].,Socias, T[1].,Bustamante, E[4].,Burzio, L[1].,Villegas, J[1,2].,[1]Andes Biotechnologies Fundación Ciencia & Vida.[2]Facultad Ciencias Biológicas Universidad Andrés Bello.[3]. Clínica Alemana.[4]. Fundación Arturo Lopez Pérez. (Sponsored by FONDEF D10i1090; CCTE-PFB16, CONICYT, Chil

Yearly, about 530.000 women are diagnosed with cervical cancer and about 50% die due the disease. Previously, we characterized a family of long non-coding mitochondrial RNAs (ncmtRNAs), named sense (S) and antisense (AS) transcripts. These RNAs are expressed in normal proliferating cells, but the AS ncmtRNA is down-regulated in tumor cells. Knock-down of AS transcript *in vitro*, using antisense oligonucleotides (ASO) induces massive cell death with hallmarks of apoptosis in tumor cells lines. The aim of this work was to evaluate the efficacy of ASO treatment in primary cell cultures obtained from biopsies of advance cervical cancer. We established two cultures, and molecular characterization show presence of HPV 16 genome, citoqueratin-17 (CK-17) and expression of $p16^{INK}$. ASO efficacy was evaluated comparing the cytotoxic effect among the antisense treatment and the drug cisplatin, using MTT assay. Moreover, the ASO treatment affects severely the tumorigenic properties, as demonstrated for strong reduction on the spheres formation assay. Xenografts model were established and ASO regimen injection of 10 doses was performed. The results of *in vivo* therapy indicate that this approach can be used as neoadjuvant therapy.

Evaluation of the expression of a family of non-coding mitochondrial RNAs throughout the progession of cervical cancer: a novel tool for diagnosis

Dadlani, K[1,2]., Villota, C[3,2].,Ávila, R[2].,López, C[2].,Zapata, L[4].,Roa, J[5].,Burzio, L[2,3].,Villegas, J[2,3].,[1]Fac. Ciencias Químicas y Farmacéuticas Universidad De Chile.[2]Andes Biotechnologies Fundación Ciencia para la Vida.[3]Fac. Ciencias Biológicas Universidad Andrés Bello de Chile .[4]Departamento de Anatomía y Patología Hospital Barros Luco Trudeau.[5]Departamento de Patología, Center for Investigation in Translational Oncology, Pontificia Universidad Católica de Chile. (Sponsored by (FONDEF D10I1090; CCTE-PFB-16 Program, CONICYT, Chile; CONICYT Scholarship For Masters Studies In Chile))

The WHO estimates that by 2020 cervical cancer will increase by 25%, being the main cause a persistent infection by Human Papillomavirus (HPV). Early detection of is key to prevent the progression of this disease. The classic diagnosis is a Papanicolaou test; however, the efficiency of this test is hampered by a high number of false negatives. We have characterized a family of Non-coding Mitochondrial RNAs (ncmtRNAs) comprised of sense (SncmtRNA) and antisense molecules (ASncmtRNAs), which show a differential expression depending on the cell proliferative status. Normal proliferating cells express both transcripts, whereas in tumor cells only the sense (SncmtRNA) transcript is detected. Cells immortalized with HPV-16 or 18 and tumor cells show a down-regulation of the ASncmtRNAs. The aim of this work was to evaluate the expression of these RNAs, through all the stages of cervical cancer, correlating it with known biomarkers, such as p16INK4A and PCNA, by *in situ* hybridization and inmunohistochemistry in tissue sections diagnosed as normal, LSIL, HSIL and invasive cervical carcinoma. Our results show a difference in the expression of the ncmtRNAs between the different grades of the disease, where the ASncmtRNA is down-regulated in 98% of cases, but normal cervical organotypic epithelial express both RNAs. We will discuss the subcellular localization of the SncmtRNA and its relationship with the disease's progression. These results suggest that the expression of these transcripts during the progression of cervical cancer can be used as a diagnostic tool

Unraveling the vitamin C-cancer paradox.

Peña, E[1]., Roa, F[1]., Gutierrez, F[2]., Muñoz, C[1]., González, M[1]., Sotomayor, K[1]., Oñate, S[3].,Vera, J[1]., Rivas, C[1]., [1]Fisiopatología, Ciencias Biológicas, Universidad De Concepción.[2]Morfo-Pathophysiology, Health Sciences, San Sebastián.[3]Especialidades, Medicina, Universidad De Concepción. (Sponsored by Fondecyt Grants 1130842 And 1140429)

The role of vitamin C in cancer remains highly controversial. Human breast cancer tissue contain high vitamin C levels compared with normal tissue, while in colon and endometrial cancers a low vitamin C content is associated with aggressive tumors. The proposed use of megadoses of vitamin C to treat cancer is in contrast with evidence that cancer cells supplemented with vitamin C are highly resistant to chemotherapy. It has also been proposed that the expression level of vitamin C transporters may be used to develop vitamin C-based therapeutic strategies. We performed a critical analysis of the capacity of breast cancer cells to transport vitamin C, determined the kinetic properties and molecularly identified of the transporters involved, analyzed their expression levels and subcellular distribution, and performed a comprehensive immunohistochemical analysis of the transporters and markers currently used to stratify breast cancer in three hundred samples of human breast cancer. Our results indicate that breast cancer cells express several vitamin C transporters that are highly compartmentalized, and that breast cancer progression is accompanied by major changes in vitamin C transporter expression and subcellular distribution. These findings may explain in part the current controversy in understanding the role of vitamin C in cancer. Fondecyt Grants 1130842 and 1140429. edpena@udec.cl

Antisense therapy in genitourinary cancer: Non coding mitochondrial RNAs as target

Borgna, V[1].,Lobos-Gonzales, L[2].,Avila, R[2].,Rivas, A[3].,Lopez, C[2].,Socias, T[2].,Burzio, L[2].,**Villegas, J[4].**, [1]Fundaciòn Ciencia & Vida, Andes Biotechnologies S.A., Facultad de Medicina, Universidad Andres Bello.[2]Cancer Lab Andes Biotechnologies S.A. - Fundación Ciencia & Vida.[3]Instituto de Ciencias Biomedicas, Facultad de Medicina, Universidad De Chile.[4]Ciencias Biologicas Universidad Andrés Bello- Andes Biotechnologies S.A. - Fundaciòn Ciencia & Vida. (Sponsored by (INNOVA-CORFO 12IEAT-16317-CCTE-PFB16, CONICYT))

Bladder cancer (BC) and renal cell carcinoma (RCC) ranks second and seventh in frequency among malignancies of the genitourinary tract respectively. More than 50% of the patients with primary invasive tumor develop metastases and chemotherapy regimens do not represent efficient treatment for these diseases. We previously described a novel family of long non coding mitochondrial RNAs (ncmtRNA), named sense and antisense. These transcripts are differentially expressed according to the proliferative status of cells. Normal proliferating cells express both transcripts, tumor cells down-regulate the expression of the ASncmtRNA. The aim of this study was to evaluate *in vitro* and *in vivo* the therapeutic efficacy of antisense oligonucleotide (ASO) treatment. ASO treatment of human bladder tumor cell lines, and murine renal adenocarcinoma (RenCa), show that after 48h a strong effect over cell viability, about 60%, is obtained in all cell lines. Also, ASO treatment induces a strong mitochondrial membrane depolarization and all cell lines exhibit over 50% Annexin-V and TUNEL-positive cells. ASO treatment also affects tumorigenic properties of these cells, both invasion and anchorage-independent growth are practically abolished after treatment. *In vivo*, a xenograft sub cutaneous model for UM-UC-3 cells and syngeneic orthotropic model for RenCa were established. A regimen of 10 ASO injection every other day was carried out, obtaining a strong reduction in tumor growth, suggesting that ASO therapy can be a novel neoadjuvant therapy.

The DEAD box polypeptide 3 protein (DDX3) regulation of human T-cell leukemia virus type 1 (HTLV-1) IRES translation depends on the cellular context.

Astudillo, A[1]., Olivares, E[1].,López-Lastra, M[1].,[1]Laboratorio de Virología Molecular, Escuela de Medicina, Pontificia Universidad Católica de Chile . (Sponsored by FONDECYT 1090318 Y P09/016-F Iniciativa Científica Milenio Del Ministerio De Economía, Fomento Y Turismo.)

The HTLV-1 full-length mRNA can initiate translation by using an internal ribosome entry site (IRES). The mechanism of IRES recognition by the eukaryotic translation initiation machinery is unknown. The RNA helicase DEAD box protein polypeptide 3 (DDX3) has been shown to modulate translation initiation of the mRNA of other retroviruses known to harbor an IRES such as that of the Human Immunodeficiency Virus type I (HIV-1). These observations prompted us to evaluate the effect of DDX3 on the activity of the HTLV-1 IRES in different cellular contexts. To this end, a vector expressing the DDX3 protein was co-transfected in HeLa and HEK 293T cells together with the bicistronic vector dl HTLV-1 IRES or dl HIV-1 IRES, harboring the HTLV-1 or HIV-1 5'UTR in their intercistronic region, respectively. A vector expressing GFP and was used as a control. Expression of DDX3 and GFP was confirmed by Western Blotting. Results showed that DDX3 protein enhanced HTLV-1 IRES-dependent translation initiation in HEK 293T cells, but not in HeLa cells. Based on these findings we conclude that DDX3 regulation of HTLV-1 IRES mediated translation initiation is dependent on the cellular context.

Expression and subcellular co-localization of BRCA1 mutants and BARD1 in breast cell lines.

Herrera, C[1]., Díaz, S[1].,Faúndez, P[1].,Pérez, E[1].,Carvallo, P[1].,[1]Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile. (Sponsored by FONDECYT 1120200, CONICYT)

The main function of BRCA1 is to orchestrate DNA double strand break repair by homologous recombination. This function is enhanced by its interaction with BARD1, which promotes the nuclear localization/retention of BRCA1 by masking its nuclear export signal. Our aim is to study the molecular mechanisms that explain the abnormal cytoplasmic localization of BRCA1 in breast cancer, analyzing the expression and localization of BRCA1 and different forms of BARD1 (BARD1a and β) lacking the BRCA1 interaction domain. We used breast cancer cell lines HCC1937, and T47D and one non-tumor cell line MCF10A. We studied mRNA expression of BARD1 and its isoforms, through RT-PCR, and BRCA1 and BARD1 protein expression by western and immunofluorescence. BARD1 full mRNA had similar expression in the three cell lines, and its isoform BARD1β was expressed at higher levels in tumor than non-tumor cell line. In addition, HCC1937 cell line was transfected with BRCA1 WT and BRCA1 mutants to evaluate the subcellular localization and co-localization with BARD1. BRCA1 WT and c.3936C>T mutant were localized in the nucleus. Mutant c.306_307insA was seen perinuclear. BARD1 was expressed in the nucleus and cytoplasm in all cell lines This suggests that mutant c.306_307insA is not being localized and/or retained in the nucleus by BARD1.The study of molecular causes of BRCA1 localization in cell lines will allow us to better understand subcellular localization of BRCA1 in tumors. FONDECYT 1120200 CONICYT

Tumorigenic potential role of CXCR3A splicing variant in papillary thyroid cancer development through RET receptor transactivation

Martínez, R[1]., Fischer, M[1].,Arbulo, D[1].,Kalergis, A[2].,González, H[1].,Urra, M[1].,[1]Departamento de Cirugía Oncológica, Facultad de Medicina, Pontificia Universidad Católica De Chile.[2]Departamento de Genética Molecular y Microbiología,, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile.

Papillary thyroid cancer (PTC) has a high rate of lymph node metastasis. Signaling mediated by chemokine receptors has been implicated in this metastatic spread pattern. Indeed, we have shown that CXCR3 expression is increased in PTC, suggesting that its signaling pathway may be involved in tumor development. CXCR3 receptor has two splicing variants. In PTC, we have shown increased mRNA of the proliferative variant (CXCR3A) whereas the proapoptotic isoform CXCR3B is downregulated. Blot assays showed increased expression of both. Based on these findings we investigated if the isoforms expression pattern of CXCR3 and their chemokine signaling is involved in thyroid tumorigenesis. CXCR3A was overexpressed in TPC-1 (RET/PTC1 cancer cell line) but CXCR3B remained constant. A blocking CXCR3 antibody as well as a CXCR3 antagonist reduced cell growth by 70% in Nthy (normal thyroid cell) but only in 30% in TPC-1 cells. This indicates that CXCR3 expression correlates with proliferative responses in thyroid cells, probably by enhancing CXCR3A signaling. Consistently, Nthy-CXCR3A transfectants showed higher proliferation rate demonstrating that CXCR3A overexpression promotes cell growth. In Nthy cells, inhibition of RET signaling strongly reduced cell growth and CXCR3 antagonist prevented RET phosphorilation induced by CXCL10, a CXCR3 ligand. These results suggest that CXCR3A proliferative signaling and RET transactivation by CXCR3 receptor could contribute to thyroid tumor development.

The co-repressor RCO-1 modulates circadian gene expression and metabolic compensation of the clock in *Neurospora crassa*

Olivares-Yañez, C[1]., Larrondo, L[1].,[1]Millennium Nucleus for Fungal Integrative and Synthetic Biology and Departamento Genética Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica De Chile. (Sponsored by CONICYT, GO-21100309, Fondecyt 1131030, MN-FISB NC120043.)

N. crassa is a model organism for the study of circadian clocks, which control endogenous rhythms of different processes, including gene expression. In order to identify new components mediating the latter process we conducted a genetic screen, identifying potential candidates, among which we found rco-1. RCO-1 is the orthologue of the *S. cerevisiae* Tup1, a key co-repressor in yeast. Therefore, we sought to further evaluate the role of RCO-1 in the control of circadian gene expression in *N. crassa*. FRQ is a central component of the circadian clock in *Neurospora*, and its rhythmic expression is a key feature. Using bioluminescent transcriptional and translational reporter fusions we evaluated frq expression in this mutant observing that although its expression is rhythmic, period length and amplitude are severely affected. Moreover, in the absence of RCO-1 we observed a defect on metabolic compensation: at high glucose concentrations the period of the clock decreases. Analysis of RCO-1 target genes revealed that RCO-1 plays an important role for their circadian expression. These results indicate a dual role for this co-repressor: maintenance of proper circadian period and control of the rhythmic expression of several ccgs. In addition, through mass-spec and Co-IP we identified transcriptions factors and proteins involved in chromatin remodeling that physically interact with RCO-1, which can lead us to dissect the mechanics of action of this transcriptional regulator both at the core-clock as well as in output pathways.

Oral Session 5 Molecular Cell Biology II

Identification of transcriptions factors directly regulated by the histones acetyltransferase GCN5 in *Arabidopsis thaliana*

Aquea, F[1]., Sewell, J[2].,Long, J[2].,[1]Genética Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica De Chile.[2]Molecular, Cell and Developmental Biology Department University of California Los Angeles. (Sponsored by FONDECYT INICIO Nº 11130567 And PUC Project UHC0717)

GENERAL CONTROL NON-REPRESSIBLE 5 (GCN5) appears to be an important histone acetyltransferase required for gene expression involved in many development pathways in plants and animals. Mutations in *Arabidopsis thaliana* GCN5 (AtGCN5) show various pleiotropic defects as a consequence of affecting the activity meristem activity. Although AtGCN5 plays an essential role in chromatin modification and transcriptional regulation, its mode of action is still not understood. Proteins involved in chromatin remodelling control the development of plants and animals through directly regulating the expression of specific developmental transcription factors. In this work, we have identified a set of potential direct target genes of AtGCN5 through a combination of chromatin immunoprecipitation/DNA sequencing (ChIP-Seq) and genome-wide transcriptional profiling using RNA-seq. This analysis revealed that AtGCN5 control directly the expression of genes involved in metabolic process, nutrient transport and transcription. Among these targets, we identified 7 transcription factors belonging to different families. Almost all of them have not been characterized in plant. Using a genetic and chemical approach, these transcription factors have been validated as direct targets of AtGCN5. Functional analysis will reveal the role of these transcriptions factors in plant development and their genetic interaction with AtGCN5 in *Arabidopsis thaliana*.

Jasmonate signaling pathway is activated during salt stress-induced inhibition of root growth by a COI1 and proteasome-mediated degradation of JAZ repressors in *Arabidopsis*

Acevedo, O[1]., Miranda, G[1].,Vergara, P[1].,Figueroa, P[1].,[1]Escuela de Biotecnología, Facultad de Ciencias, Universidad Santo Tomás. (Funded By FONDECYT 1120086)

Salinity is considered a severe abiotic stress affecting irrigated croplands. Jasmonates (JAs) are important regulators of plant development and responses to abiotic stress. Our previous studies have shown that *JAZs*, JA-responsive genes encoding for negative regulators of JA mediated responses, were induced by salt stress in *Arabidopsis* roots through a COI1-dependent pathway. Using *JAZ1p::GUS* plants we showed that *JAZ1* promoter activity increased after 6-h of salt treatment, particularly in the elongation and meristematic zone of the *Arabidopsis* roots. In addition, JA sensor plants expressing a fusion JAZ1-GUS protein which is destabilized by JA, showed a decreased reporter activity in the root tip after 6-h of salt exposure by a mechanism dependent on the 26S proteasome; thus linking salt stress response with increased JA levels in roots. However, the outcomes of JA signaling activation by salt are unknown. Therefore, we investigated a putative role of JA in inhibiting root growth during salt stress by performing time-lapse imaging of JA-insensitive (*coi1-1*) and WT seedlings to measure root growth rate in a 24-h time period. We observed that *coi1-1* root growth inhibition by salt stress was significantly lower when compared with WT plants after 6-h of treatment. Taken together, these results uncover a previously unknown crosstalk between salt-triggered stress response and JA signaling pathway controlling root growth in *Arabidopsis*.

A fight between two clocks: the effect of circadian regulation in the modulation of the plant-pathogen (*Arabidopsis thaliana-Botrytis cinerea*) interaction.

Hevia, M[1]., Canessa, P[1].,Müller, H[1].,Larrondo, L[1].,[1]Millennium Nucleus for Fungal Integrative and Synthetic Biology and Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile. (Sponsored by CONICYT, FONDECYT 1131030, Millennium Nucleus NC120043.)

Circadian clocks are cellular time-telling machineries that regulate biological rhythms in gene expression, physiology and behavior in such way that they occur at precise times of day. Circadian regulation allows organisms to anticipate predictable daily changes and it has been shown that the clock´s plant anticipates its defense response at dawn. This concept has never been evaluated in pathogens, like fungi, because the only fungus in which a clock has been molecularly studied is non-pathogenic. Therefore, we have characterized a functional circadian clock in the fungal phytopathogen *Botrytis cinerea*, which ranks as the second most important according to its economic and scientific importance. We demonstrated that BcFRQ1, BcWCL1 and BcWCL2 proteins are part of the circuitry of an oscillator: a reporter between BcFRQ1 and Luciferase oscillates under constant conditions and under temperature cycles. Rhythms in *bcfrq1* and BcFRQ1 anticipate cyclical-environmental changes, a key characteristic of circadian behavior. We observed an impaired infection process using Botrytis´ clock mutant strains. Finally, we demonstrated that the outcome of the plant- pathogen interaction varies with the time of day, confirming that the Botrytis´ clock is largely controlling the process. These results confirm for the first time the existence of a circadian clock in a pathogen, putting forward the concept that fungal clocks can synchronize key elements of pathogenesis.

Polycystin 2 is required for stress-induced activation of autophagy

Criollo, A[1]., Hill, J[2].,[1]Instituto de Investigacion en Ciencias Odontologicas, Faculty of Odontology, Universidad De Chile.[2]Internal Medicine, Medicine, UT Southwestern Medical Center.

Autophagy is a process of intracellular protein and organelle recycling, critical to numerous forms of stress.Polycystins are integral membrane proteins localized to the membranes, primary cilia, and ER. Mutations in the genes encoding PC1 or PC2 (*pkd1*, *pkd2*) are implicated in a number of human diseases, including polycystic kidney disease. Importantly, the activities of mTOR and AMPK, two major regulators of autophagy, are dysregulated in these diseases. We hypothesized that PC2 regulates autophagy.The gene PC2 was selectively silenced in myocytes by crossing mice expressing Cre driven by the MHC promoter with mice harboring a floxed *pkd2* (PC2F/F). Deprivation induced accumulation of autophagic vacuoles in both WT and PC2 F/F mice. However, this phenomenon was attenuated in animals $PC2^{-/-}$.Studies *in vitro* using myocytes and fibroblasts revealed that knock down of PC2 inhibited autophagic induction elicited by starvation. Whereas starvation induced inactivation of mTOR and activation of AMPK, PC2 knockdown did not affect AKT or AMPK activity. As the ER participates in the formation of autophagic vacuoles, we evaluated the intracellular distribution of PC2 and its interaction with proteins involved in the autophagic machinery. We found that PC2 co-localized with ER markers and immunoprecipitated with BCN1. We demonstrate that PC2 is required for induction of autophagy *in vitro* and *in vivo* in a manner independent of both mTOR and AMPK. PC2 is localized to the myocyte ER, where it interacts with BCN1. These data suggest that PC2 is a novel regulator of autophagy

Transcriptional and phenotypic analysis of the plasticity of the circadian clock of *Neurospora crassa.*

Goity, A[1]., Larrondo, L[1].,[1]Millennium Nucleus for Fungal Integrative and Synthetic Biology and Departamento Genética Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica De Chile. (Sponsored by CONICYT, GO-21120421, FONDECYT 1131030, MN-FISB NC120043.)

Circadian rhythms are generated at a cellular level by a transcriptional-translational negative feedback loop, where the negative element is capable of inhibiting the activity of the positive elements that controls its expression. These rhythms are self-sustained and in constant conditions they exhibit periods close to 24 hours. In Neurospora the negative element is the protein FRQ, encoded by the gen *frequency* (*frq*) and the positive element is the White Collar Complex (WCC), composed of the transcription factors White Collar 1 (WC-1) and White Collar 2 (WC-2), where WC-1 is also a photoreceptor. These elements, in association with the protein FRH, form the core oscillator, which is capable of receiving, via the input pathways, environmental cues such as light, by the light-dependent transcriptional activation of WC-1 acting on the *frq* promoter. The oscillator is responsible for transmitting the time information to various biological processes such as growth and metabolism (output), in part, through a hierarchical arrangement mediated by transcription factors which ends in the rhythmic expression of genes controlled by the clock (*ccg*s). To challenge the current understanding of *frq* transcriptional regulation, and the importance of the transcriptional networks associated to the central oscillator, we have engineered a hybrid oscillator, rewiring existing basic components, in order to evaluate its ability to generate and maintain rhythms. We have also tested the capacity of this synthetic oscillator to respond to external environmental perturbations.

Intragenic cytosine methylation and its role in transcription regulation

Ramos, M. P.[1]., Wijetunga, N.[1].,McLellan, Andrew[1].,Suzuki, Masako[1].,Greally, John[1].,[1]Genetics, John Greally, Albert Einstein College of Medicine.

Despite the general link between promoter DNA methylation and transcriptional supression, many inactive genes maintain unmethylated promoters. Since the role of cytosine methylation outside promoters is less understood, we analyzed its levels from a genome-wide perspective. We paradoxically found that euchromatic regions have higher levels of DNA methylation compared to heterochromatin, especially in the bodies of actively transcribed genes. Therefore, a major driver of DNA methylation is transcriptional activity.To understand the biological role of cytosine methylation, we treated HEK293T cells with the DNMT1 inhibitor 5-aza-2'-deoxycytidine (5-aza-CdR). DNA demethylation was dose dependent and persisted as an "imprinted" memory following drug withdrawal, despite metabolic recovery of the treated cells compared to control. We identified that 5-aza-CdR treatment is mainly targeting actively transcribed, euchromatic regions of the genome, but limited changes in protein-coding and lncRNA transcriptional levels were observed. Strikingly, we did find evidence of the activation of intragenic promoters. The subset of differentially expressed genes shared interesting features, being enriched for cellular pathways altered in cancer, suggesting that 5-aza-CdR treatment could be predisposing non-tumorigenic cells to promote neoplasia. These new insights conform a foundation for understanding the therapeutic and toxic effects of DNA demethylating drugs in clinical use, establishing the importance of understanding global DNA methylation patterns beyond promoter regions.

JMJD1B regulates the processing of cytosolic histone H3

Saavedra, F[1,2]., Alvarez, F[1,2].,Rivera, C[1,3].,Li, J[4].,Forné, I[4].,Zack, G[5].,Imhof, A[4].,Almouzni, G[5].,Loyola, A[1].,[1]Laboratorio de Epigenética y Cromatina Fundación Ciencia & Vida.[2]Facultad de Ciencias Biológicas Universidad Andrés Bello.[3]Facultad de Ciencias Químicas y Farmacéuticas Universidad De Chile.[4]Adolf-Butenandt-Institute LMU University of Munich..[5]Nuclear Dynamics and Genome Plasticity Intitut Curie. (Funded By FONDECYT 1120170, Basal Project PFB16 And Doctoral Fellowship CONICYT 21140346)

The processing of the cytosolic histone H3 includes the translation of the mRNA by ribosomes and the maturation of the protein through a cascade that comprises at least four steps with different complexes. In this maturation cascade, the histones acquire their correct folding and posttranslational modifications (PTMs) previous to their incorporation into the nucleus. In contrast to the highly modified nucleosomal histone H3, the cytosolic histone has only a few modifications, being the most abundant of them the monomethylation of the lysine 9 (H3K9me1). Our previous results suggested that the establishment of the mark H3K9me1 occurs during the translation of the histone, while associated to the ribosome, in a SetDB1 dependent mechanism. Interestingly, we have also detected the histone H3K9 demethylase JMJD1B associated to the ribosome. To understand the importance of this demethylase in the processing of newly synthesized histones, we investigated the role of this enzyme. Our results suggests that JMJD1B is not just a histone demethylase, but is also a regulator of the processing of the cytosolic histone H3.

Second messengers are required for lateral root formation induced by the endocytic trafficking modulation in *Arabidopsis thaliana*

Rubilar-Hernández, C[1].,Pérez-Henríquez, P[1].,Norambuena, L[1].,[1]Centro Biología Molecular Vegetal, Facultad de Ciencias, Universidad De Chile. (Sponsored by FONDECYT 1120289; CONICYT Doctoral Fellowship.)

Lateral roots are plant organs in charge of absorbing nutrients from soil. Different external stimuli promote lateral root formation (LRF) in plants through the auxin nuclear receptor SCF-TIR1/AFBs-dependent signaling pathway. However, evidence suggests the LRF can be modulated also by a signaling pathway with independence of this receptor. We have shown that the endocytic trafficking is a positive regulator of LRF by a SCF-TIR1/AFBs-independent signaling pathway by means of using the chemical Sortin2. We have utilized Sortin2 to find key cellular processes of the SCF-TIR1/AFBs-independent signaling pathway on LRF. We have found that important activities for protein trafficking as phosphatidilinositide 3- and 4-kinase are required for the LRF induced by Sortin2. Also, we have observed that Sortin2-induced LRF is suppressed by inhibiting calcium ion entry to the cytoplasm. Interestingly, calcium entry is not required for exogenous auxin-induced LRF, suggesting that the signaling pathways induced by exogenous auxin and Sortin2 are different. Moreover, Sortin2 stimulates the production of reactive oxygen species in primary roots. Consequently, LRF induced by Sortin2 is suppressed by inhibiting NADPH oxidase activity suggesting that reactive oxygen species production participates on Sortin2-induced LRF. Therefore, the results suggest that phosphatidylinositol phosphate metabolism, calcium ion and reactive oxygen species are key factors on the LRF by a SCF-TIR1/AFBs-independent signaling pathway induced by the endocytic trafficking modulation in *A. thaliana*.

Oral Session 6 Gene Expression and Molecular Cell Biology

A reverse genetics approach unveils a link between cell fusion pathways and the circadian clock in Neurospora

Montenegro-Montero, A[1]., Larrondo, L[1].,[1]Millennium Nucleus for Fungal Integrative and Synthetic Biology and Departamento de Genética Molecular y Microbiología Pontificia Universidad Católica De Chile. (Sponsored by FONDECYT 1131030, MN-FISB NC120043)

Circadian clocks control the daily expression of numerous genes in different organisms. The fungus *N. crassa* has one of the best-understood circadian systems and plenty is known about the molecular basis of its core oscillator. How the information is transmitted out of the oscillator to control overt rhythms however, is largely unknown. By using a luciferase-based system, we show that the expression of several bZIP transcription factors (TFs) is controlled by the circadian clock in Neurospora. We used protein binding microarrays to examine the binding specificities of Neurospora TFs to characterize regulatory networks (known as output pathways) controlling rhythmicity of these genes. Our data suggested that a Zn cluster TF, previously associated with cell fusion, was involved in bZIP rhythmicity, but careful genetic analysis suggested that a secondary, unmapped mutation, present in the deposited TF KO, was in fact responsible for both the fusion defect and the circadian phenotype. Additional genetic studies unveiled that global defects in cell fusion result in altered rhythmicity, revealing for the first time a link between this process and the clock. Whole genome sequencing identified the mutation responsible for the phenotype and we further found the alteration to be present in other mutants of the KO collection. Our study highlights a novel process involved in output pathways in addition to providing a cautionary note on the use of KO strains from deletion collections.

Cis-regulatory elements involved in species specific transcripcional regulation of the rat SVCT1 gene

Muñoz, A[1]., Villagrán, M[1].,Gatica, M[1].,Mardones, L[2].,Maldonado, M[1].,Rivas, C[1].,Oñate, S[3].,Vera, J[1].,[1]Fisiopatología, Ciencias Biologicas, Universidad De Concepción.[2]Departamento de Ciencias Básicas y Morfología, Facultad de Medicina, Universidad Católica de la Santísima Concepción.[3]Departamento de Especialidades, Medicina, Universidad De Concepción. (Sponsored by Fondecyt Grants 1130842, 1140429; Conicyt Doctoral Fellowship; AT-24100130 Scholarship)

Ascorbic acid is transported into cells by the sodium-coupled vitamin C transporters (SVCTs). Recently, we obtained evidence of differential regulation of SVCT expression in response to acute oxidative stress in cells from species that differ in their capacity to synthesize vitamin C, with a marked decrease in SVCT1 mRNA and protein levels in rat hepatoma cell lines that was not observed in human hepatoma cells. To better understand the regulatory aspects involved, we performed a structural and functional analysis of the proximal promoter of the *SVCT1* rat gene. We cloned a 1.5 kb segment containing the proximal promoter of the *SVCT1* rat gene, generated partial constructs of decreasing sizes by deletion with restriction enzymes and mutant promoters by site-directed mutagenesis, and analyzed their capacity to direct the transcription of a reporter gene after transfection in rat and human hepatoma cells. This was complemented with the co-expression of transcription factors whose consensus binding sequences are present in the SVCT1 promoter. This analysis revealed the presence of two regulatory sites crucial for the transcriptional activity of the promoter of the rat *SVCT1* gene, sites that are absent in the promoter of the human *SVCT1* gene. These findings suggest that the regulation of the vitamin C metabolism in humans may differ from that of species capable of synthesizing vitamin C de novo. Fondecyt Grants 1130842, 1140429; Conicyt Doctoral fellowship; AT-24100130 scholarship. alemunoz@udec.cl

Polyglucosan molecules does not induce changes in the stability and integrity of an in vitro blood-testis barrier

Villarroel-Espindola, F[1]., Guinovart, J[2].,Concha, I[1].,Slebe, J[1].,[1]Bioquímica y Microbiología, Ciencias, Universidad Austral De Chile.[2]CIBER de Diabetes y Enfermedades Metabólicas (CIBERDEM) Institute for Research in Biomedicine (IRB Barcelona), España. (Sponsored by FONDECYT 3130449 And 1141033)

Sertoli cells are responsible to support the spermatogenesis and seminiferous tubules architecture by the conformation of the blood-testis barrier (BTB). The functionality of BTB determines the viability and differentiation of male germ cell. We reported that the glycogen accumulation in testis of transgenic animals overexpressing a superactive form of glycogen synthase (KIN-saGS) enhances the apoptosis of pre-meiotic cells. Now we extend and amplified this work on the effects of glycogen storage in GC1 and Sertoli cells (42GPA9 cell line) and the mechanism behind the pro-apoptotic activity induced. By spectrophotometric analysis, we found that glycogen synthesized in both cell lines—by saGS expression or by activation of endogenous GS—is poorly branched. In addition, the cleaved caspase3 detection suggests that apoptosis induced by glycogen affects GC1 but not 42GPA9 cells. Furthermore, we analyzed the effects of glycogen deposition during the establishment of an in vitro BTB. The results using Evans blue dye showed that 42GPA9 cells do not lose their capacity to generate an impermeable barrier and the expression of connexin43 (Cnx43), occludin (Occl), and ZO1 proteins were not affected by glycogen accumulation. In the KIN-saGS mice, the distribution of actin and ZO1 showed closed tubules, affecting the viability of male germ cells but not the Sertoli cells. These results confirm that the accumulation of glycogen has a selective effect in testis.

Deconstructing the transcriptional compensatory system of the *Neurospora crassa* circadian Clock.

Muñoz-Guzmán, F[1]., Caballero, Valeria[1].,Larrondo, Luis[1].,[1]Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile. (Sponsored by FONDECYT 1131030, MN-FISB NC120043.)

All organisms in which circadian clocks have been characterized exhibit a common molecular circuit, which is based on a positive element able to activate the expression of a negative one that then represses the action of the former, inhibiting its own expression. This system is capable of sustain oscillations under several external perturbations, but the compensation mechanisms remain unknown. Studies in mammals, *Drosophila* and *Arabidopsis* have shown some new transcriptional inputs supporting the central molecular circuit, by modulating the expression of the core-clock components (CCC). In Neurospora crassa the clock central circuit has been well characterized but the participation of other transcriptional networks (TN) in this system are still unknown. In our lab, we are trying to find these novel TNs controlling the CCC in *N. crassa*. Thus, we have defined a set of transcriptional regulators (TR) that modulate circadian features. In addition, we have dissected the transcriptional units of the molecular circuit, trying to identify cis-elements impacting the robustness of the system. Finally, combining the results of both strategies with information of DNA binding preference for over 50% of the TRs available in *N. crassa*, we constructed a Global Transcriptional Network, with the TR interactions and their possible target genes. Arranging all the information in a comprehensive way, we are starting to decode the TNs behind the central circadian oscillator of Neurospora and the perturbations to which this compensatory system is capable of responding.

The establishment of the post-translational modification H3K9me1 on cytosolic histones H3

Alvarez, F[1,2].,Saavedra, F[1,2].,Ugalde, V[1].,Díaz-Célis, C[1].,Rivera, C[1,3].,Forné, I[4].,Imhof, A[4].,Loyola, A[1].,[1]Laboratorio de Epigenética y Cromatina Fundación Ciencia & Vida.[2]Facultad de Ciencias Biológicas Universidad Andrés Bello.[3]Facultad de Ciencias Químicas y Farmacéuticas Universidad de Chile.[4]Adolf-Butenandt-Institute LMU University of Munich. (Funded By FONDECYT 1120170, Basal Project PFB16, CONICYT Doctoral Fellowship 21140324.)

The processing of cytosolic histones H3 and H4 includes the translation of mRNA by ribosomes and the translocation of the synthesized proteins to the nucleus. This process occurs in a cascade of maturation that ensures the correct folding and the establishment of post- translational modifications. This maturation cascade is mediated by the association of histones with different chaperones and enzymes in six cytosolic complexes. In contrast to the highly modified nucleosomal histone H3, the cytosolic histone H3 has only a few modifications. It is acetylated at the lysines 14 and 18 (H3K14K18ac) and monomethylated at the lysine 9 (H3K9me1). Given that the H3K9me1 mark is present in the first complex of the maturation cascade, we suggest that this mark is imposed in the ribosome. Experiments from our group show that the histone methyltransferase SetDB1 is stably associated to the ribosome. Moreover, histone H3 associated to ribosomes presents the mark H3K9me1. Interestingly, when we knocked down SetDB1, we observed a decreased of the methyltransferase activity and the H3K9me1 mark on the ribosome. Our results suggest that the modification H3K9me1 is established while histone H3 is being translated, prior to its association with the cytosolic complexes.

Leucocyte treatment with the anti-cancer drug etoposide induces specific genomic aberrations in *RUNX1* gene

Schnake, N[1]., Álvarez, P[1].,Gutiérrez, S[1].,[1]Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad De Concepción. (Sponsored by FONDECYT Nº 1130697)

Secondary leukemia is a severe side effect that affects about 10% of cancer patients treated with etoposide, a topoisomerase II inhibitor. Genomic aberrations associated with acute myeloid leukemia, such as chromosomal translocation (8;21), are often found in those patients. However, the exact mechanism behind the generation of that particular type of cancer is largely unknown. Intravenous administration of etoposide implies that peripheral blood cells are the first cells that come in contact with the drug. Interestingly, previous results show that treatment with etoposide induces specific damage in leucocites DNA at intron 5 of *RUNX1*, one of the genes involved in chromosomal translocation (8;21). The exact nature of this damage is unknown, therefore we hypothesize that treatment with etoposide generates specific genomic aberrations in *RUNX1* intron 5. To test this hypothesis, we assesed genomic aberrations using inverse genomic polymerase chain reaction on DNA from samples of peripheral blood treated with clinically relevant concentrations of etoposide. Surprinsingly, our results show that genomic aberrations in leucocites *RUNX1* due to treatment with etoposide are not random, and suggest that chromosomal translocations associated with leukemia can be induced in these cells.

The role of the nuclear cap-binding complex on gene expression from the full-length unspliced HIV-1 genomic RNA

García, F[1]., Rojas, B[1].,Pereira, C[1].,Soto-Rifo, R[1].,[1]Programa de Virologia, ICBM, Medicina, Universidad De Chile. (Sponsored by Fondecyt 11121339)

The nuclear cap-binding complex (CBC), composed of the CBP20/80 heterodimer, binds to the m^7G cap structure of the RNA polymerase II (RNAPII) transcripts and plays critical roles during transcription, splicing, nuclear export, non sense-mediated decay and the pioneer round of translation. As a typical RNAPII-transcribed mRNA, the HIV-1 genomic RNA (gRNA) is capped and recruits the CBC. However, and in contrast to the vast majority of the cellular mRNAs, the HIV-1 gRNA retains its introns resulting in the inability to use the classical NXF1-mediated mRNA export pathway. Nevertheless, the viral protein Rev serves as an adaptor between the gRNA and the cellular export factor CRM1, thus allowing nuclear export of the viral mRNA through a non-canonical pathway. To date, its unknown whether the recruitment of the CBC to the gRNA cap impacts viral gene expression as has been shown for classical cellular mRNAs exported by NXF1. Here, we show that overexpression of CBP80 but not CBP20 stimulated both cytoplasmic accumulation and translation of the gRNA. Interestingly, this role of CBP80 required the CBP20-mediated cap binding activity of the CBC and more importantly, was dependent on the presence of the Rev protein. Finally, we used molecular modelling to gain insights into the mechanism by which Rev binds to CBP80 in the context of the CBC. Together, our data suggest that the Rev protein binds to the gRNA cap-associated CBC allowing the formation of a specific viral nuclear export mRNP that favours both, cytoplasmic accumulation and translation of the HIV-1 gRNA.

DNA methylation profile of metallothionein and vitellogenin gene in response to endocrine disruptors in liver of *Cyprinus carpio*

Stolzenbach, M[4]., Valenzuela, G[1].,Vidal, G[2].,Laengst, G[3].,Figueroa, J[4].,Kausel, G[4].,[1]Instituto Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral De Chile.[2]Insituto Acuicultura, Sede Puerto Montt, Universidad Austral De Chile.[3]Biochemistry III, Faculty Biochemistry and Preclinical Medicine, University Regensburg, Germany.[4]Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral De Chile. (Acknowledgment: DAAD 50750108, DID-UACh PEF2012-3, Fermelo S.A.)

Environmental changes affect gene expression that we tested in the sentinel organism for endocrine disruptors, the common carp *Cyprinus carpio*. Previously we had shown by RT-qPCR in total liver RNA with respect to mock treated fish in response to Zn treatment a significant increase of metallothionein (MT involved in protection against heavy metals and oxidants) and in 17-b-estrogen (E2) treated male carp an increase of vitellogenin (VTG, precursor of the egg yolk usually present only in female). Next we analyzed DNA methylation patterns in *mt* and *vtg* gene sequences extracted from carp genome database with primers spanning CpG-rich regions. Genomic DNA from liver of Zn or E2 and control treated carp was fragmented by sonication, incubated with bisulfite, amplicons were cloned and 8 up to 14 independent sequences were analyzed from each region. In the 25 Cs analyzed in 748bp stretch of mt promoter a decrease of methylation in the region of 15 C proximal to ATG was revealed in Zn respect to control treated carp, which suggests that methylation pattern is affected in response to metal stimulus. Clearly, analyses of 8 Cs in 428bp in the *vtg* promoter, more sequences with unmethylated C were found in E2 treated carp contrasting complete methylation in that region in male controls.

Therefore, here we report for the first time that DNA methylation dynamics seem to play a role in the epigenetic-based mechanisms mediating transcriptional response of *mt* and *vtg* expression to Zn or E2 treatment in liver of *C. carpio*.

Poster Sessions

Posters Session I

1) Role of the conserved HXE and NXXE motifs in the ADP-dependent glucokinase from *Thermococcus litoralis*

Abarca, J[1]., Ramirez-Sarmiento, C[1].,Merino, F[2].,Rivas-Pardo, J[3].,Guixé, V[1].,[1]Departamento de Biología, Ciencias, Universidad De Chile.[2]Departament of Cell and Developmental Biology Max Planck Institute for Molecular Biomedicine.[3]Departamento de Biología, Ciencias , Universidad De Chile. (Sponsored by Fondecyt 1110137)

Some archaea use a modified Embden-Meyerhof pathway that employs an ADP-dependent glucokinase. These kinases belong to the ribokinase superfamily and possess highly conserved motifs such as the NXXE and HXE motifs related to binding of two divalent cations. One cation is present in the metal-nucleotide complex, while the other is a regulatory cation that modulates the energy difference between the transition and ground states. The information available suggests that the NXXE motif is related with the catalytic metal, whereas the regulatory metal binds to the HXE motif. We evaluated the role of E279 and E308, associated to HXE and NXXE motifs respectively, in the kinetics parameters of the glucokinase from *Thermococcus litoralis* by site-directed mutagenesis. The E279Q and E279L mutants showed 5 and 3-fold increase in the Km for MgADP compared to the wild type enzyme, and 80 and 100-fold decrease in k_{cat}, respectively; surprisingly, a 30 and 400-fold increase in the Km for glucose for E279Q and E279L was observed. The E308Q mutant showed 100-fold increase in the Km for MgADP with no change in the Km for glucose. HXE-related mutants maintained the inhibitory effect of increasing concentrations of free metal on the glucokinase activity, which is not observed in the E308Q mutant. These results indicates that E308 is important for MgADP binding to allow phosphate transfer and that this binding step would be critical for subsequent binding of the regulatory metal to the HXE motif.

2) Chronic exposure to insulin alters mitochondrial morphology and function in cardiomyocytes

Acuña, F[1]., Riveros, C[1].,Vasquez-Trincado, C[1].,Quiroga, C[1].,Lavandero, S[1,2].,[1]Advanced Center for Chronic Diseases (ACCDiS) and Center for Molecular Studies of the Cell (CEMC), Faculty of Chemical and Pharmaceutical Sciences & Faculty of Medicine, University of Chile.[2]Department of Internal Medicine, Southwestern Medical Center University of Texas. (Supported By FONDAP 15130011 (SL), FONDECYT 3120220 (CQ), FONDECYT 1120212 (SL). CVT Holds CONICYT PhD Fellowship)

Resistance to insulin is the most important factor for the development of metabolic and cardiovascular diseases. The underlying mechanism behind this pathology involves desensitization of the insulin signaling pathways, generating a defect on glucose transporter translocation to the plasmatic cell membrane. Cardiac mitochondrial function is particularly affected by this condition due to high energy cellular demand of this tissue. To evaluate the relationship between mitochondrial function, morphology and insulin resistance, cultured rat cardiomyocytes were treated with insulin 10 nM for 24 h and re-exposed for 30 minutes insulin 10 nM again. The data showed that this short pulse of insulin increased Akt and FoxO1 phosphorylation in control cardiomyocytes, but this effect was completely abolished after the long-term exposure to insulin. Similar results were found with insulin-dependent ^{3}H-2D-glucose uptake, ROS production and mitochondrial membrane potential. This chronic exposure to insulin also altered mitochondrial morphology and proteins involved in mitochondrial fusion (Mfn-2, Opa-1) and fission (Drp-1) processes. Collectively, these results show that an *in vitro* chronic exposure to insulin down-regulated insulin signaling pathway, promotes mitochondrial fission and decreased energy metabolism in cultured cardiomyocytes.

3) Transcriptomic analysis of handling stress response of the red cusk eel (*Genypterus chilensis)* skeletal muscle.

Aedo, E[1]., Aballai, V[1].,Fuentes, E[2].,Gallardo-Escarate, C[3].,Molina, A[2].,Valdés, J[1].,[1]Laboratorio de Bioquímica Celular, Facultad de Ciencias Biológicas. Interdisciplinary Center for Aquaculture Research (INCAR), Universidad Andrés Bello.[2]Laboratorio de Biotecnología Molecular, Facultad de Ciencias Biológicas. Interdisciplinary Center for Aquaculture Research (INCAR), Universidad Andrés Bello.[3]Laboratory of Biotechnology and Aquatic Genomics, Facultad de Ciencias Naturales y Oceanográficas. Interdisciplinary Center for Aquaculture Research (INCAR), Universidad De Concepción.
(Sponsored by CONICYT/FONDAP/15110027)

Introduction: The red cusk-eel (*Genypterus chilensis*)has been considered one of the endemic species of greatest farming potential in Chile. Recently, we generated a reference transcriptome of *G. chilensis* under handling stress, a model of stress inherent in aquaculture. In this work, we evaluated handling stress effects on gene expression associated with skeletal muscle growth of *G. chilensis.* **Material and Methods:** Total RNA was extracted from skeletal muscle of juvenile red cusk-eel under control and stressed conditions, and sequenced by Illumina technology. Reads were mapped onto the previously generated reference transcriptome using CLC genomic workbench software version 7.0.3. RNAseq analyses were validated through RT-qPCR. **Results:** We identified 122 differentially expressed genes *in silico* associated to catabolic process under response to handling stress. We found that the ubiquitin–proteasome participants: *FOXO, Atrogin-1* and *26S proteasome regulatory subunit S1;* theautophagy-lysosomal participants: *Atg 5*, *Atg 16* and the inhibitors of protein synthesis: *4EBP-1* and *REDD-1* were over-expressed under stress. **Discussion:** Our results indicate that handling stress up-regulated expression gene of important atrophy signaling pathways in *G. chilensis*. This study is the first step towards the comprehensive understanding of the influence of stressful farming conditions on the molecular and endocrine mechanisms that control growth in red cusk-eel, a non-model fish species.

4) Acute stress controls FMRP levels and its activity through AKT-mTOR and MAPK ERK1/2 pathways in rat hippocampus

Aguayo, F[1]., Rojas, P[1,2].,Márquez, R[1].,Pacheco, A[1].,García Perez, A[1].,García-Rojo, G[1].,Muñoz Llanos, M[1].,Fiedler, J[1].,-[1]Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad De Chile.[2]Escuela de Química y Farmacia, Facultad de Medicina, Universidad Andrés Bello. (Sponsored by FONDECYT1120528)

Mechanisms of synaptic plasticity that involve remodeling of neural circuitry require de-novo protein synthesis. In neurons, some mRNAs are transported to dendrites and translated by highly regulated process. In this context, the Fragile X Mental Retardation Protein (FMRP) is a RNA-binding protein which transports mRNAs associated to protein complexes. Besides, phosphorylated FMRP acts as translational repressor, activity modulated by S6K1 kinase related to AKT-mTOR and MAPK ERK1/2 pathways. We evaluated the effect of acute restraint stress on p-FMRP protein levels and the activation of AKT-mTOR and MAPK ERK 1/2 pathways in hippocampi extracts. Adult male rats were stressed during 0.5 or 2.5 h and sacrificed immediately after restraint session or 1.5, 6 and 24 h post stress. During stress procedure an increase in AKT-mTOR pathway activity was observed, in contrast to the delayed activation of ERK1/2 observed at 6 h post stress. Further, FMRP levels increased during restraint procedure, and 24 h post stress a rise in p-FMRP was observed which could be related to ERK1/2 activation and/or variation in FMRP amount. Finally, the Fmr1 transcript was augmented 24 h post stress, in parallel with the reduction in FMRP protein levels, suggesting compensatory mechanism.

5) Effect of histone demethylase- and methyltransferase- inhibitors over replication and transcriptional activity of the Hepatitis B Virus.

Alarcon, V[1].,Muñoz, F[2].,Rubio, L[3].,Hernández, S[5].,Flores, Y[4].,Villanueva , R[5].,Loyola, M[1].,[1]Laboratorio de Epigenética y Cromatina Fundación Ciencia & Vida, Universidad San Sebastián.[2]Laboratorio de Epigenética y Cromatina Fundación Ciencia & Vida, Universidad Andrés Bello .[3]Laboratorio de Epigenética y Cromatina Fundación & Vida, Universidad Andrés Bello.[4]Laboratorio de Epigenética y Cromatina Fundación Ciencia & Vida.[5]Laboratorio del Virus de la Hepatitis, Facultad de Ciencias Biológicas, Universidad Andrés Bello. (Sponsored by ANILLO ACT119, FONDECYT 1120170, Basal PFB16, USS 2011-005-R.)

Infection with the Hepatitis B virus (HBV) is a major cause of liver disease and hepatocellular carcinoma. The HBV genome replicates its DNA in the nucleus of the infected hepatocytes via a covalently closed circular DNA (cccDNA). The cccDNA is the responsible for the persistent infection of hepatocytes and it serves as template for the transcription. It forms a minichromosome associated with cellular proteins such as histones. Our hypothesis is that the viral chromatin plays a regulatory function and modulates transcription and replication of HBV. Our work has focused on the role of histone demethylases and methyltransferases over the viral replication and transcriptional activity. We used an *in vitro* culture system reflecting the HBV viral replication cycle to investigate the role of the histone H3K4 and H3K9 demethylase Lysine Specific Demethylase 1 (LSD1) and the histone H3K4 methyltransferase complex COMPASS. Our results suggest that both enzymes contribute to the HBV life cycle progression, opening novel strategies for developing new therapies against the viral replication.

6) Identifying new transient receptor potential melastatin 8 (TRPM8): From sequence to structure.

Alegría-Arcos, M[2].,Almonacid , D[1,2].,Latorre, R[1].,Gonzalez-Nilo, F[2,1].,[1]Centro Interdisciplinario de Neurociencias de Valparaíso (CINV) Universidad de Valparaíso.[2]Center for Bioinformatics and Integrative Biology (CBIB), Facultad de Ciencias Biológicas, Universidad Andrés Bello. (Sponsored by All Authors Would Like To Acknowledge CINV (Millennium Science Initiative 09-022-F) And FONDECYT 1131003.)

Transient receptor potential melastatin 8 (TRPM8) is a cation channel whose activation depends on cold temperature and the lipid composition of its membrane as well as other factors, such as voltage and the presence of menthol. TRPM8 channel is a member of TRPM family that has been shown highly expressed in prostate tumours. From the above arguments, TRPM8 channel has gained relevance in the last decade. We have created a sequence similarity network (SNN) to identify new TRPM channel families. This network allows determining the relationships among sequences of a large number of proteins, focusing in identifying new TRPM8 channels. Moreover, we investigated the activation of TRPM8 channel by phosphatidylinositol-4,5-biphosphate (PIP2). Since no channel structure of this family has been solved, in this work, we built a model for a single TRPM8 representative member. Then, we performed molecular docking and molecular dynamics studies allowing us to characterize the binding sites of PIP2 in TRPM8 channel.

7) Facile and cost-effective detection of saxitoxin exploiting aptamer structural switching

Alfaro, K[1]., Bustos , P[2].,Bustos , P[2].,O´Sullivan, C[3].,Conejeros, P[4].,[1]Centro de Investigación y Gestión de Recursos Naturales, Ciencias, Universidad De Valparaíso.[2]Centro de Investigación y Gestión de Recursos Naturales, Facultad de Ciencias, Universidad De Valparaíso.[3]Chemical Engineering Universitat Rovira i Virgili .[4]Centro de Investigación y Gestión de Recursos Naturales Universidad De Valparaíso. (Sponsored by Fondecyt 11110050 And PIA ACT 1108.)

A simple method to detect saxitoxin (STX), one of the main components of the paralytic shellfish poison from red tides, was developed. By using a next generation dye for double stranded DNA we were able to differentiate fluorescence from STX-aptamers when exposed to different concentrations of STX, suggesting a change in aptamer folding upon target binding. The developed method is extremely rapid, only requiring small sample volumes, with quantitative results over the concentration range of 15 ng/ml to 3 µg/ml of STX, with a detection limit of 7.5 ng/ml.

8) Skeletal muscle plasticity induced by seasonal acclimatization in carp involves differential expression of rRNA and molecules that epigenetically regulate its synthesis

Fuentes, E.,Ramos, I[1].,Zuloaga, R[1].,Nardocci, G[1].,Fernandez De La Reguera, C[1].,Simonet, N[1].,Fumeron, R[1].,Valdes, J[1].,Molina, A[1].,**Alvarez, M**[1]., [1]Departamento Ciencias Biologicas, Facultad Ciencias Biologicas, Universidad Andrés Bello. (Sponsored by FONDECYT 1120873, CONICYT/FONDAP/15110027)

Considerable evidence indicates that epigenetic mechanisms can arise as a consequence of environmental inputs (temperature, pH, salinity, etc.). Consequently, the contribution of epigenetic mechanisms in the transcriptional regulation of genes during adaptation process is an exciting topic in molecular biology. Thus, epigenetics emerges as one of the molecular strategy through which an organism is able to adapt to a dynamic environment. Accordingly, fish appear as an ideal model for investigating the organism-environment interface and the process that emerge from it. In the present work, we have evaluated seasonal expression of a set of epigenetic factors (TTF-1, Tip5, NML, SUV39H1, SIRT1), histone variants (H2A.Z, mH2A1, mH2A2, H2A.Z.7) and molecular factors involved in ribosomal biogenesis (ubf1, p80-coilin, nucleolin) in skeletal muscle from *C. carpio*. The results showed that mRNA contents in muscle for *ttf-I, tip5, sirt1, nml, suv39h1, mh2a1, mh2a.z*, and *nuc* were up-regulated during winter in comparison with summer, whereas the mRNA levels of *mh2a2, ubf1,* and *p80-coilin* were down-regulated. Our findings show that environmental clues are regulating the expression of rRNA and molecules that epigenetically regulate its synthesis in carp muscle. By suppressing this major ATP-consuming biosynthetic processes process during winter, growth is shut down in order to save on energy expenditure. This process could therefore be an adaptive evolutionary strategy that allows this organism to survive in fluctuating habits.

9) Evaluation of the epigenetic response generated by stress management in red ling fish *(Genypterus chilensis)*

Sepulveda , J[1,2].,Ramos, I[1,2].,Salazar, M[1].,Kruger, G[1,2].,Valdes, J[1,2].,**Alvarez, M[2,1].**, [1]Interdisciplinary Center for Aquaculture Research (INCAR), Víctor Lamas 1290, PO Box 160-C, Concepcion, Chile.[2]Departamento Ciencias Biologicas, Facultad Ciencias Biologicas, Universidad Andrés Bello. (Sponsored by CONICYT/FONDAP/15110027, FONDECYT 1120873)

In the last decades, the Chilean aquaculture industry has grown exponentially, mostly due to the increased production of salmonids species. Nevertheless, the intensive farming of salmonids has generated a large number of health problems and environmental impact. The aquaculture diversification constitutes a strategic challenge that must be oriented towards the culture of new native species of high economic potential. Actually, a fish with farming potential is the red ling *(Genypterus chilensis)*, which occurs naturally along the Chilean coast but has not achieved its successful farming in captivity.

In order to determine which factors influence the farming of the red ling fish, we evaluated its changes at physiological and molecular level mediated by handling stress conditions. First, we have identified and isolated the partial coding sequences of some mayor epigenetics factors. Then, we evaluated blood cortisol and transcriptional expression of some regulatory factors (AROS) and histone variants (mH2A2 and H2A.Z) in fish undergoing handling stress. Our results show significant changes in both, blood cortisol and transcriptional level of the epigenetic factors, confirming an epigenetic response triggered by stress in red ling. With this, we want provide original knowledge at the molecular level of how epigenetics response is implemented in a new fish model and in this way to achieve a rational management of new native species in farming conditions.

10) Differential protein-protein interactions of the human CCAAT/enhancer-binding protein beta (hC/EBPβ) transcription factor isoforms.

Amigo, R[1]., Arriagada, A[1].,Rodríguez, F[1].,Valenzuela, N[1].,Hepp, M[1].,Gutiérrez, J[1].,[1]Departamento Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas , Universidad De Concepción. (Sponsored by CONICYT, FONDECYT/Regular 1130818)

C/EBPβ is a transcription factor involved in several cellular processes, such as proliferation and differentiation. Three isoforms (C/EBPβ1, C/EBPβ2 and C/EBPβ3) can be produced by translation at alternative initiation codons of a unique mRNA, being C/EBPβ1 the longest isoform and C/EBPβ3 the shortest one. Since C/EBPβ3 lacks the whole transactivation domain, this isoform has been usually found to act as transcriptional repressor, while C/EBPβ1 and C/EBPβ2 have been found to act as activators or repressors, depending on the cellular context. Previous work in our laboratory, coupling GST pull-down to Mass Spectrometry analysis, suggested the existence of differential interaction between C/EBPβ isoforms with certain High Mobility Group (HMG) proteins and histone H1. To this respect, competition between HMG proteins and histone H1 for chromatin binding sites has been described, with a deep impact on chromatin dynamics and gene expression. In our present study we sought to confirm these differential interactions through GST pull-down coupled to detection with specific antibodies, using nuclear extracts from different cell lines and affinity-purified recombinant HMG proteins. In addition, interaction with the XPC complex, a complex commonly linked to DNA repair but with a role in transcriptional regulation recently described, was also analyzed. Our studies confirm a significantly higher affinity of the C/EBPβ long isoforms for HMG proteins.

11) Biological activity *in vitro* of active peptides present in the venom of chilensis *Brachistostermus*

Arán, T[1]., Araya, P[1].,Olivares, H[2].,Catalán, A[1].,Sagua, H[1].,Gonzalez, J[1].,Neira, I[1].,Ordenes, K[1].,Orrego, P[1].,Araya, J[1].,Rojas , J[1].,[1]Tecnología Medica, Facultad de Ciencias de la Salud, Universidad De Antofagasta.[2]Biomedico, Facultad de Ciencias de la Salud, Universidad De Antofagasta.

Today, animal poisons and toxins are being considered as innovative biotechnological tools for prospection of news drugs or as models for the synthesis of new drugs for therapeutic use in humans drugs. Within the different biotechnological studies from different regions of the world, highlighting the analyzes venom of snakes and spiders which evaluated their potential antimicrobial and / or anti-cancer.In the present study we evaluated the venom of *Brachistostermus chilensis* (Kraepelin, 1911) of a scorpion endemic family *Bothriuridae* Chile, located between the region of Valparaíso and the Metropolitan region. Dehydrated *B. chilensis* venom, at an approximate concentration of 25 mg/mL , was resuspended in buffer 50 mM ammonium acetate pH 7.0 and centrifuged at 10,000 rpm for 15 minutes to eliminate insoluble debris. The supernatant was applied to a column of CM-Sephadex C-25 equilibrated with the same buffer, the retained proteins were eluted with the buffer containing 0.6M NaCl and 0.25M NaCl. Then each was evaluated for antibacterial activity and also their hemolytic activity. Six protein peaks were obtained of which three of them have some antimicrobial activity on *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*. None of the proteins present hemolytic activity.

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12) Analysis of the metal-specificity of the poplar Kunitz trypsin inhibitor (PdKTI3) through complementation in yeast *(Saccharomyces cerevisiae)*.

Gutiérrez, A[1].,Arancibia, S[2].,Silva, C[2].,**Guerra, F[1].**, [1]Instituto de Ciencias Biológicas Universidad de Talca.[2]Escuela de Ingeniería en Bioinformática Universidad de Talca. (Sponsored by FONDECYT - Grant Number 11110214)

The expression of genes encoding Kunitz-type trypsin inhibitors (KTI) has been related to the defensive response of plants to multiple biotic and abiotic stresses. Particularly, Populus deltoides KTI3 gene (*PdKTI3)* is highly up-regulated when poplars are exposed to different copper stress conditions, suggesting its participation in the tolerance against the negative effect induced by the excess of this metal. In order to confirm that role and establishing its metal-specificity, we carried out a series of complementation assays in different *Saccharomyces cerevisiae* strains. Three mutant strains (delta CUP2, GSH1 and ZRC1) were transformed using expression vectors (Invitrogen Gateway cloning system) carrying (or not) the PdKTI3 gene. Transformant cells were grown individually in solid culture media enriched with distinct doses of copper, cadmium, zinc and nickel. A differential effect, in terms of complementation, was observed under the assessed treatments, which depended on the strains and metals. Results indicated the ability of PdKTI3 to reverse the metal-sensitivy of some mutant strains and its specificity to copper and cadmium.

13) New formulation based in anti-atrophic peptides and dendrimers for the treatment of skeletal muscle atrophy: Molecular dynamics studies.

Araya Durán, D[1,2]., Pacheco, N[2].,Márquez-Miranda, V[1,2].,Ratjen, L[2,1].,Cabello-Verrugio, C[3].,Morales, G[3].,Rivera, J[3].,González-Nilo, F[2,1].,[1]Nanomedicine Fraunhofer Chile Research.[2]Center for Bioinformatics and Integrative Biology (CBIB), Ciencias Biológicas, Universidad Andrés Bello.[3]Laboratorio de Biología y Fisiopatología Molecular, Ciencias Biológicas, Universidad Andrés Bello. (Sponsored by V.M.M Thanks CONICYT Doctoral Fellowship. This Work Was Supported By Fraunhofer Chile Research, Innova-Chile CORFO (FCR-CSB 09CEII-6991), FONDECYT 1120380, AFM 16670, UNAB DI-280 And Anillo Científico ACT1107.)

Angiotensin 1-7 (Ang1-7) is a bioactive heptapeptide with beneficial effects on the treatment of circulatory system, skeletal muscle and nervous system diseases. We have determined that Ang1-7 decreases skeletal muscle atrophy. However, due to their instability, peptides cannot be efficiently administered. Therefore, it is required to develop a new method of delivery that increases the half-life of the peptide and improve its bioavailability in target tissues. Dendrimers have emerged as promissory vehicles to protect and transport a wide range of bioactive molecules. In this work, we explore the use of neutral, non-cytotoxic PAMAM–XX dendrimer as carrier of Ang1-7 peptide by molecular dynamics simulation (MD). Results showed that, over 60 ns of MD simulation, peptide-binding capacity of the dendrimer was 2:1 molar ratio, in agreement with experimental data. MD analysis also revealed the capacity of PAMAM-XX to protect Ang1-7 and form stable complexes. Peptide coverage ability of the dendrimer was around 50 and 65%. Furthermore, electrophoretic mobility shift assay demonstrated that PAMAM-XX increases the stability of the peptide, thus it can act as an efficient carrier.

14) HHeterologous expression in various cell lines and partial characterization of the human hexose transporter GLUT12

Arce, R[1]., Ojeda, L[1].,Cuevas, A[1].,Reyes, A[1].,Pérez, A[1].,[1]Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral De Chile. (Sponsored by FONDEF D11I1131, FONDECYT 1130386, DID-UACh S-2013-20)

Glucose is a major source of energy in eukaryotic cells; thus, its transport into the cells is critical. As a hydrophilic molecule, it is unable to cross the cell membrane by simple diffusion and requires specialized carrier proteins, the GLUT transporters, who facilitate it to pass through membranes. Fourteen isoforms of the GLUT carriers has been described in human. Since little is known regarding the functional characteristics of human GLUT12 transporter, we probe the expression of this transporter in different cell lines, to identify an expression system appropriate to define kinetic parameters such as K_M and V_{max} for their substrates and to test the effect of transport inhibitors. We try several cell lines including HEK293, CHO-K1, MDCK cells, and various transfection protocols as alternatives for expressing GLUT12. While with some we managed to detect expression in cell membranes by Western blot, their low levels prevented us to perform the functional expression of transport. Finally, GLUT12 functional expression was attained in a system of stably-transfected MDCK cells. Although the level of expression was modest, it was enough to demonstrate that GLUT12 transports hexoses and that this transport is sensitive to inhibition by quercetin and cytochalasin B, known blockers of the classic GLUT transporters

15) Analysis of the binding site and pathway of the specific drug A1899 into the potassium channel TASK-1

Arévalo, B[1].,Ramirez, D[1].,Rinné, S[2].,Decher, N[2].,Gonzalez, W[1].,[1]Centro de bioinformática y simulación molecular, Facultad de Ingeniería, Universidad De Talca. [2]Institut für Physiologie und Pathophysiologie, Fachbereich Medizin Philipps-Universität Marburg. (Sponsored by Fondecyt 1140624)

Two-pore domain potassium (K_2P) channels are expressed as functional dimers in the central nervous system, cardiovascular system, genitourinary system and gastrointestinal system. They are related with several pathologies in humans. Thus, members of this family have emerged as molecular candidates for the action of pharmacological agents. The K_2P channel TASK-1 is an important modulator of multiple sclerosis and in 2011 a highly-selective blocker of TASK-1, named A1899, was discovered. A1899 acts as an open-channel blocker and binds to residues forming the wall of the central cavity. In 2012 the first crystal structures of K_2P channels were published. Electron density maps revealed two open lipid cavities or fenestrations, one on each side of the dimer, that expose the central cavity to the membrane. We constructed homology models of TASK-1, based on crystal structures of the recently crystallized K_2P channels –with and without fenestrations- and studied the specific binding site of A1899 by means of molecular docking. Our results suggest that A1899 travels through the inner cavity to the center of the pore to block the currents of TASK-1.

16) Flux Balance Analysis of *Escherichia coli* grown on acetate: The effect of NAD(P)H production by Isocitrate Dehydrogenase

Armingol, A[1]., Matsuda, L[1].,Brescia, I[1].,Cabrera, R[1].,[1]Laboratorio de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Chile. (This Work Was Supported By FONDECYT-Chile 1121170.)

During the growth of *Escherichia coli* on acetate as sole carbon source, isocitrate dehydrogenase (ICDH) contributes over 90% of total NADPH production. ICDHs can be classified in dependence of their specificity: those using NADP to oxidize isocitrate (such as the *E. coli* ICDH) and the NAD-dependent ICDHs. Zhu *et al.* (2005) have previously studied the replacement of the endogenous *E. coli* ICDH by a NAD-dependent form and its effect over the growth rate, by generating an icd^{NAD} strain. According to their results, they postulated the use of NADP by *E. coli* ICDH as consequence of an evolutionary event that allowed growing on acetate. However, changes in the metabolic flux distribution have remained unassessed. In this work, metabolic flux distributions were analysed *in silico* for ICDH modified strains of *E. coli*: icd^{NAD} and icd^{NAD} ΔpntAB (having this last one the deletion of membrane transhydrogenase PntAB). By using COBRA, we integrated the iJO1366 *E. coli* model with the acetate consumption rates obtained experimentally from batch cultures. Our results showed the adaptive potential of *E. coli* under different genetic backgrounds. The simulations predicted the activation of malic enzyme and flux increments through the glyoxylate shunt, gluconeogenesis and the oxidative branch of the Pentoses Phosphate pathway. Thus, our work allows a deeper understanding of the genome-scale metabolic adaptations of *E. coli* grown on acetate.

17) Structure-Function Relationship of an 11-residues Antimicrobial Lysine (Lys) Homopeptide and their Alanine (Ala) and Proline (Pro) Scans.

Aróstica, M[1]., Ojeda, C[2].,Marshall, S[3].,Rojas, *R[1].,Carvajal, P[4]., Guzman , F[2].,[1]Instituto de Química Pontificia Universidad Católica De Valparaíso.[2]Núcleo de Biotecnología Curauma and Fraunhofer Chile Research Foundation Pontificia Universidad Católica De Valparaíso.[3]Instituto de Biología Pontificia Universidad Católica De Valparaíso.[4]Escuela de Alimentos Pontificia Universidad Católica De Valparaíso. (Proyect Fondecyt 1140926 *Beneficiario Beca Postgrado PUCV 2014)

Synthetic homopeptides have been used as models for structural and functional studies of peptides due to the relative simplicity of data interpretation. In a previous work, we demonstrated that an 11-residue Lys homopeptide was capable of inhibiting the growth of a broad spectrum of bacteria. To gain a better understanding of the inhibition mechanism of this homopeptide, we synthesized it and their Ala and Pro scanning analogs by Fmoc solid-phase peptide synthesis. The antimicrobial activity was analyzed by the microbroth dilution method using four types of bacterial targets. The structural variations of the peptides were determined by circular dichroism spectroscopy in different medium, DMPG and *E. coli* membrane extract. Confocal laser scanning microscopy (CLSM) was used to study the interaction of the homopeptide with *E. coli* to assess the ability of the peptide to alter the permeability of the bacterial membrane. In general, Lys residues located at either C-terminal or at the N-terminal end of the 11-residue Lys homopeptide played a key role for bacterial inhibition, contrary to Lys residues located at the center of the peptide, as demonstrated by Ala and Pro exchanges. Ala substitutions at any position of the homopeptide produced a more pronounced change in the PPII structure compared to Pro substitutions. However, the PPII conformation remained stable in the presence of different solvents, DMPG and *E. coli* extract. CLSM observations revealed that the 11-residue Lys was able to penetrate the bacterial membrane by pore formation and kill the bacteria.

18) Use of bioinformatics tools for designing degenerated primers against enzyme families in the pathway of lysine synthesis in Corynebacterium glutamicum.

Asenjo, F[1]., D, Almonacid[1].,F, Sepulveda[1].,L, Álvarez[2].,[1]Center for Bioinformatics and Integrative Biology (CBIB), Facultad de Ciencias Biológicas, Universidad Andrés Bello.[2]Bionanotechnology and Microbiology Lab, Facultad de Ciencias Biológicas, Universidad Andrés Bello. (We Acknowledge Funding From GRANT Regular UNAB DI-476-14/R).

Our aim is to identify prokaryotic organisms containing homologous enzymes to those involved in lysine synthesis in *Corynebactrium glutamicum*. To that end we designed degenerate primers for the enzymes that make up this metabolic route. From the protein sequences of these enzymes, obtained from the Uniprot database, blastp was used to identify all other proteins that belong to the same families in NCBI's nr and env_nr databases. Since these searches identify a large number of proteins, the CDHIT software was used to generate various clusters according to sequence identities. For each cluster a representative protein was selected. All representatives were subjected to a blastp against all other representatives and the results were displayed as sequence similarity networks in the Cytoscape program. In these networks, nodes are protein sequences and edges are Boston E-values. Functional information is mapped to each node from Uniprot. Iso-functional clusters of proteins were identified in the networks, and those containing the enzymes of interest were chosen for the generation of degenerate primers with the following software: iCODEHOP, Primaclade and Hyden. Finally, the generated primers were evaluated using OligoCalc and mFOLD.

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19) Tumor suppression by caveolin-1 is linked to the inhibition of autophagy and mitochondrial dysfunction in cancer cells

Ávalos, Y[1,2]., Bravo-Sagua, R[1,2].,Troncoso, R[2].,Lavandero, S[2].,Quest, A[1].,[1]Laboratorio de Comunicaciones Celulares, Centro de Estudios Moleculares de la Célula (CEMC), Centro de Estudios Avanzados en Enfermedades Crónicas (ACCDiS), Programa de Biología Celular y Molecular, Instituto de Ciencias Biomédicas (ICBM), Facultad de Medicina, Universidad De Chile.[2]Laboratorio de Transducción de Señales Moleculares, Centro de Estudios Moleculares de la Célula (CEMC), Centro de Estudios Avanzados en Enfermedades Crónicas (ACCDiS), Facultad de Ciencias Químicas y Farmacéuticas, Universidad De Chile. (Sponsored by FONDAP 15130011, ACT1111 (AFGQ,SL), Fondecyt 1130250 (AQ) And CONICYT Post-doctoral Training (RT) And PhD (YA,RB) Scholarships.)

Caveolin-1 (CAV1) is a scaffolding protein that suppresses tumor formation by B16F10 mouse melanoma cells in C57BL6 mice. Whether tumor suppression by CAV1 is linked to inhibition of autophagy, a lysosomal degradation process, is not known. Here, we evaluated whether CAV1 modulates autophagy in B16F10 melanoma cells and MDA-MB-231 breast cancer cells by determinating autophagic flux (western blotting), cell death (flow cytometry) and oxygen consumption (oxygen electrode) under basal and starvation conditions in the presence or absence of the autophagy inhibitor chloroquine (CQ). The *in vivo* role of CAV1 and autophagy were tested by evaluating tumor formation in mice treated with CQ and by western blotting analysis of p62, a protein that is degraded by autophagy. Expression of CAV1 decreased the autophagy marker LC3-II and oxygen consumption in basal and starvation conditions. Moreover, the inhibition of autophagy with CQ increased cell death in CAV1-expressing cells as compared with controls. *In vivo* assays showed that the volume of tumors formed by B16F10(mock) cells was reduced to that observed for B16F10(Cav-1) cells when treated with CQ. Also, p62 levels were elevated in tumors formed by B16F10(Cav-1) cells. These results suggest that CAV1-mediated inhibition of autophagy and mitochondrial function contribute to the tumor suppressor role of CAV1.

20) Safety and selectivity of antisense approach in cervical cancer: non coding mitochondrial RNAs as therapeutical targets

Ávila, R[1]., Villota, C[1,2].,Lobos-Gonzales, L[1].,Socias, T[1].,Burzio, L[1].,Villegas, J[1,2].,[1]Andes Biotechnologies Fundación Ciencia & Vida.[2]Facultad Ciencias Biológicas Universidad Andrés Bello. (Sponsored by FONDEF D10i1090; CCTE-PFB16, CONICYT, Chile)

Advanced cervical cancer is a severe disease that not offers efficient therapeutic options, therefore, new therapies with low side effects constitute a permanent challenge. We characterized a family of non-coding mitochondrial RNAs (ncmtRNAs) consisting of sense and antisense transcripts. Normal proliferating cells express both RNAs, whereas in tumor cells only the sense transcript is expressed. The aim of this work was to evaluate the safety and selectivity of ASO treatment using SiHa and human foreskin keratinocytes (HFK) cells as models of tumor and normal cells respectively. Antisense oligonucleotides (ASO) targeting the AS transcript, triggers 60-80% of cell death in tumor cells, without affects the viability of normal proliferating cells. Moreover, the proliferation rate, assessed by DNA synthesis assay, shows that after treatment in tumor cells, is stopped but is not affected in normal cells. Double treatment has not effect over cell viability or DNA synthesis in normal cells. Finally, toxicological assay using Balb-c mice show that ASO injection do not induces an inflammatory response or adverse effects on mice organs. Altogether, these results suggest that ASO treatment is selective and safe for to be used as therapeutic option for this cancer.

21) Effect of the expression of viral proteins in cell bodies, on cross-presentation in dendritic cells, *in vitro*.

Barrientos, C[1]., Morales, J[1].,Cardozo, Y[1].,Cortez, M[1].,Montoya, M[1].,Torres , E[1].,Aranda, M[2].,Acuña-Castillo, C[1].,[1]Centro de Biotecnología Acuícola, Facultad de Química y Biología, Universidad de Santiago de Chile.[2]Facultad de Química y Biología Universidad de Santiago de Chile. (Sponsored by FONDECYT 1110734)

Immunogenic cell death (ICD) and cross priming appears to be crucial events for induction of an effective immune response against cancer. We developed a methodology for development ICD on tumor cells and evaluated whether fusion proteins of the infectious salmon anemia virus (ISAv), human respiratory syncytial virus (HRSV) or Murray Valley encephalitis virus (MVEV), (stable transfected prior the cell death induction) can stimulate DCs maturation and improve the cross priming. DCs were generated from bone marrow derived from C57BL/6 mice, under standard conditions. DCs were treated with different fusogenic CBs and their respective controls, and the expression of activation markers CD40, CD86, MHC-I and MHC-II were determined, additionally the complex formed between the SIINFEKL peptide bound to MHC-I was determined by flow cytometry. The results show that the all CBs evaluated have the ability to induce maturation of DCs in a dose dependent manner. In addition the CBs generated for cells expressing hRSV fusion protein promotes the cross-presentation in DCs. Our results suggest that the fusogenic CBs could be an attractive strategy against cancer due to facilitation in recognizing, favoring maturation and cross-presentation in DCs.

22) Subcellular localization and activity of a DNA glycosylase/AP lyase (TcNTH1) from *Trypanosoma cruzi*

Barrientos, C[1]., Ormeño, F[1].,Valenzuela, L[1].,Ponce, I[1].,Sepúlveda, S[1].,Astorga, R[1].,Cabrera, G[1].,Galanti, N[1].,[1]Programa de Biología Celular y Molecular, ICBM, Facultad de Medicina, Universidad De Chile. (Funded By: FONDECYT Project N°1130113 (N.G))

Trypanosoma cruzi is a protozoan parasite causative agent of Chagas disease, an endemic zoonosis in Latin America. The parasite presents three cellular forms which survive DNA damage caused by reactive species (ROS / RNS) in the insect vector and the mammalian host. The Base Excision Repair (BER) pathway is one of the most important repair mechanisms for DNA oxidative damage in eukaryotes. The first step of the BER pathway is the recognition of an oxidized base by DNA glycosylases. NTH1 is a bifunctional DNA glycosylase that recognizes and removes pyrimidine oxidized derivatives, to subsequently catalyze the cleavage of the DNA strand by an AP lyase activity. NTH1 of *T. cruzi* (TcNTH1) was overexpressed and purified from epimastigotes, in order to evaluate its subcellular localization and enzymatic activity. By immunofluorescence analysis TcNTH1 was found at nuclear level. On the other hand, previous results have shown that TcNTH1 does not remove the oxidized base thymine glycol. Thereby it was evaluated its cleavage capacity over an oligonucleotide containing an AP site, resulting in the excision of the apurinic-apyrimidinic (AP) substrate. Our results suggest that the TcNTH1 enzyme is an AP lyase, though we cannot discard an endonuclease activity.

23) The use of antisense oligonucleotides as an effective, selective and safe therapy for bladder cancer in the mouse model C57BL/6-MB49.

Bendek, M[1,2]., Lobos-González, L[2].,Silva, V[2].,Silva, V[1,2].,Avila, M[1,2].,Villegas, J[3,2].,Burzio, L[3,2].,Landerer, E[4,2].,[1]Escuela de Ingeniería en Biotecnología, Facultad de Ciencias Biológicas, Universidad Andrés Bello.[2]Andes Biotechnologies Fundación Ciencia & Vida.[3]Facultad de Ciencias Biológicas Universidad Andrés Bello.[4]Facultad de Medicina Universidad Andrés Bello. (Sponsored by Grants Proyecto BASAL PFB-16 And Fondecyt 11100385, Conicyt, Chile.)

Bladder cancer is the eleventh most frequent type of cancer in both sexes worldwide. The standard treatment of superficial bladder cancer is the trans-urethral tumor resection (TURB), followed by immunotherapy and/or chemotherapy. Approximately 85% of these patients develop tumor relapse. An emerging modality in the fight against bladder cancer is gene therapy, where the most used is antisense oligonucleotides (ASOs). For the development of these new therapeutic strategies, validation in models that resemble the environment of the disease is necessary. An immunocompetent orthotopic syngeneic murine model of superficial bladder cancer C57BL/6-MB49 was implemented. The animals were treated intravesically with phosphorothioate ASOs. The ASO therapy with a standard intravesical BCG treatment was compared. The bladders of the animals were surgically removed, fixed and embedded in paraffin for histological sections and evaluated by a veterinary pathologist. As research parameters muscular tumor invasion and survival was determined.Mice treated with the ASO complementary to ASncmtRNa, showed an increase in survival with a median of 86 days versus 74 days of those treated with BCG and 53 days of the group treated with an unrelated ASO. Also a decrease in the rate of muscle invasion with only 3.7% of them compared to 30% of the controls. These results suggest that the ASO therapy could be used as a neo adjuvant against bladder cancer.

24) Mifepristone-induced metabolic changes in skeletal muscle cells

Bernal, I[1,2]., Farias, M[1,2].,Vasquez-Trincado, C[1].,Navarro-Marquez, M[1].,Mellado, R[2].,Troncoso, R[1].,[1]Advanced Center for Chronic Diseases (ACCDiS), Facultad de Ciencias Quimicas y Farmaceuticas, Universidad de Chile.[2]Departamento de Farmacia, Facultad de Química, Pontificia Universidad Católica De Chile. (Sponsored by FONDECYT 11130285 (RT), FONDAP 15130011 (RT))

Mifepristone is a drug that contains anti-progestational properties, and is the only FDA-approved drug with anti-glucocorticoids actions. Mifepristone had been shown beneficial properties in Cushing syndrome and insulin resistance. Because skeletal muscle does not express progesterone receptor, effects of mifepristone in this tissue are attributed to the interaction of the drug with the glucocorticoid receptor (GR). We studied the effect of the mifepristone on metabolism in rat L6 skeletal muscle cells. Metabolism was assessed by mitochondrial membrane potential (Ψ_{mt}), oxygen consumption and ATP levels. In addition, we assessed the effect of mifepristone on insulin signaling by exposure of GLUT4 to the cell membrane, and by western blot the total and phosphorylated forms of Akt and insulin receptor (IR) were determined. Our results showed that L6 cells exposed to mifepristone for 24h decreased Ψ_{mt} and increased oxygen consumption. Moreover, mifepristone improved the insulin signaling measured as GLUT4 exofacial exposure and phosphorylation of Akt and IR. Our data suggest that mifepristone regulates basal and insulin-stimulated metabolism in L6 skeletal muscle cells.

25) Yes-associated Protein 1 (YAP1) expression is associates with attenuation of Hippo kinase core and Survivin expression in gallbladder cancer

Bizama, C[1]., García, P[1].,Riquelme, I[2].,Weber, H[2].,Espinoza, J[1].,Alfaro, F[3].,Romero, D[3].,Leal, P[2,4].,Apud, M[1].,Roa, J[5].,[1]Departamento de Anatomía Patológica, CITO, Facultad de Medicina, Pontificia Universidad Católica de Chile.[2]Departamento de Anatomía Patológica, CEGIN-BIOREN, Facultad de Medicina, Universidad de La Frontera .[3]Departamento de Anatomía Patológica, Facultad de Medicina, Pontificia Universidad Católica de Chile.[4]McKusick-Nathans Institute of Genetic Medicine, School of Medicine, Johns Hopkins University.[5]Departamento de Anatomía Patológica, CITO, FONDAP ACCDIS, Facultad de Medicina, Pontificia Universidad Católica de Chile. (Research Supported By FONDECYT 3140426, 1130204, 11130515.)

Gallbladder cancer (GBC) is a highly fatal and aggressive disease characterized by late diagnosis, poor prognosis and few therapeutic alternatives. An excellent candidate for targeted therapy in cancer is the Hippo signaling pathway that has been reported to be deregulated in different carcinomas and it is involved in multiple cellular functions that are central in tumorigenesis, including cell proliferation, apoptosis, tumor stem cell phenotype, drug resistance and metastatic potential. Here, we have characterized the expression of the main components and downstream targets of the Hippo pathway in neoplastic tissues and cell lines of gallbladder cancer. YAP1 immunohistochemical expression was seen strong and intermediate in nucleus and cytoplasm in 71% (139/195) of GBC tissues. Quantitative real time and immunobloting analyses showed a significant down-regulation of the Hippo kinase core suppressor genes (LATS2, MST1 and MST2) and up-regulation of BIRC5/survivin, a direct target of Hippo pathway. Our findings suggest that Hippo pathway contributes to gallbladder tumorigenesis and mediates its oncogenic effects through of attenuation of their suppressor core components and survivin expression.

26) The conductance rate of potassium channels is correlated to the K^+ dehydration process

Bravo, F[1]., Sepúlveda, R[1].,Diaz, I[1].,Aguayo, D[1].,Gonzalez, F[1].,[1]Centro de bioinformatica y biologia integrativa (CBIB), Ciencias Biológicas, Universidad Andrés Bello.

Potassium channels are membrane proteins able to elicit the passage of K+ across the membrane. They share a common signature sequence TVGYGD - the selectivity filter (SF) of the channel- that account for both, selectivity and high transport rates. Nevertheless, K+ channels display a high variability in their single channel conductance, ranging from 2-200pS. Given that SF is conserved among K+ channels, the rate-limiting step accounting for such differences must reside elsewhere in the conduction pathway.

In order to shine light on the latter problem, we performed non-equilibrium molecular dynamics simulations in both, a small and large conductance K+ channels Shaker and BK, applying an external electric field to different voltages +300, +600mV, and +800mV in order to characterize the outward conduction process. We found, that major differences exist in the hydration degree of the K+ ions in their progress through the conduction pathway. In BK channels, the loss of the hydration sphere occurs close to the selectivity filter while in Shaker –a small conductance channel- this step occurs at a more distant constriction of the pore, termed the PVP motif. Our results suggest, that unitary conductance is strongly modulated by the physical dimensions of the conduction pathway, and the hydration steps that an incoming K+ experiences to reach the selectivity filter. Acknowledgements: This work was supported by FONDECYT 1131003 (FGN), 1120818 (DN) and CINV (Millennium Initiative, 09-022-F).

27) Caveolin-1 and Protein kinase A regulate ER-mitochondria communication during ER stress

Bravo-Sagua, R[1]., Rodríguez, A[1].,Ávalos, Y[1].,Parra, V[2].,Quiroga, Cl[1].,Paredes, F[1].,Ortiz-Sandoval, C[3].,Simmen, T[3].,Quest, A[1].,Lavandero, S[1,2].,[1]Advanced Center for Chronic Diseases (ACCDiS) Universidad De Chile.[2]Southwestern Medical Center University of Texas.[3]Faculty of Medicine and Dentistry University of Alberta.

The endoplasmic reticulum (ER) is crucial for protein and calcium homeostasis. In response to stress, the ER increases its contacts with mitochondria (sites termed MAMs) in order to facilitate calcium transfer between both organelles and thus boost metabolism. Caveolin-1 and Protein kinase A (PKA) regulate mitochondrial metabolism and dynamics, respectively; however, whether these signaling molecules regulate MAM function is unknown.

ER stress increased ER-to-mitochondria calcium transfer in HeLa cells, as assessed by fluorescence microscopy, and oxygen consumption, suggesting an increase in organelle cross-talk. Furthermore, ER stress induced mitochondrial elongation, which correlated with PKA relocation to mitochondria and inhibition of the mitochondrial constriction GTPase DRP1. PKA inhibitor H89 prevented these changes, highlighting their dependence on PKA. Caveolin-1 overexpression, on the other hand, inhibited PKA redistribution, MAM remodeling and the increase in mitochondrial calcium uptake and cell bioenergetics.

Our results suggest that PKA is a positive regulator of ER-mitochondria cross-talk, while Caveolin-1 inhibits this effect by preventing PKA redistribution.

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28) Hijacking of the Complement System by *Clostridium difficile spores* for entry into the host cells

Brito, C., Castro, P[1].,Paredes, D[1].,[1]Ciencias Biológicas , Ciencias Biológicas , Universidad Andres bello.

Clostridium difficile is a Gram-positive, anaerobic, spore-former, which has become the main cause of diarrhea associated with adult health care. Spores of *C. difficile* are considered morphotype of transmission and persistent of the infection, and colon it's their ecological niche. Episodes of *C. difficile*-associated infections are successfully treated with antibiotics, however recurrence rates caused by the remaining spores reaching up to 60%. En this study we explored interactions between *C. difficile* spores and intestinal epithelial cells, also with macrophages and complement component which mediate immune response against *C. difficile*. Here, we report that complement components mediate phagocytosis of spores in murine cell line Raw 264.7 and internalization in intestinal epithelial cell line Caco-2. In vitro experiments show that opsonin complement component C3 is deposited on the surface of the spores and also the recognition protein of the classical via of the complement system, C1q. Infection in absence of this component decreases phagocytosis and internalization of the spores in Raw 264.7 and Caco-2 respectively. These findings suggest a novel mechanism for pathogen entry into host cells as well as new evidence to explain persistence of the spores in the colon.

29) PcACE1 from *P. chrysosporium*: a bifunctional transcription factor

Bull, P[1]., Silva, M[1]., Rojas, V[1]., Villegas, C[1]., Salinas, F[1]., Larrondo, L[1]., [1]Depto Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile. (Sponsored by Funded By VRI 2761-043, VRI 2761-043-81, And Núcleo Milenio FISB NC 120043)

Copper is a key element in living organisms. Too much copper is toxic, wheras too little is insufficient for living. In *S. cerevisiae*, two transcription factors regulate intracellular copper concentration; ACE1 at high (above 10 µM) and MAC1 at very low copper (below 10 nM). ACE1 contains 3 Cys motifs similar to **C**X**C**Xn**C**X**C/H** in the amino terminal. On the other hand, MAC1 contains 1 Cys motif at the amino and 2 at the carboxy terminal. We isolated PcACE1 from *Phanerochaete chrysosporium.* It is the ortholog of ACE1; it functionally complements at high copper a yeast strain lacking a functional *ace*1. PcACE1 presents five Cys motifs in its coding sequence, 3 at the amino terminal, as ACE1, and two at the carboxy terminal, as MAC1, being the first transcription factor to contain such high number. Recently we have demonstrated that at high copper, single Cys to Ser mutations in motifs 2 and 3 are crucial for PcACE1 *in vivo* transcriptional function, whereas those from motifs 4 and 5 have 70-90 % functional activity compared to 100% WT PcACE1, suggesting that it could have MAC1-like transcriptional activity. In this work we analyzed if PcACE1 also shows functional activity at very low copper. We introduced by recombination in the genome of *S. cerevisiae mac*1Δ strain, a *ctr*1 promoter linked to *lac*Z reporter gene. MAC1 binds to Ctr1 promoter. We transformed this strain with WT PcACE1, and determined β–galactosidase activity at very low copper. The results show that PcACE1 has functional activity, indicating that it is active at both high as well as low copper.

30) Primary astrocytes under pro-inflammatory conditions inhibit neurite outgrowth in an αvβ3 integrin- and Syndecan-4-dependent manner

Burgos, F[2].,Quest, A[1,3].,Leyton, L[2,3].,[1]Laboratory of Cellular Communication, Center for Molecular Studies of the cell (CEMC), Institute of Biomedical Sciences (ICBM), Facultad de Medicina, Universidad de Chile.[2]Laboratory of Cellular Communication, Center for Molecular Studies of the cell (CEMC), Biomedical Neuroscience Institute (BNI), Institute of Biomedical Sciences (ICBM), Facultad de Medicina, Universidad de Chile.[3]Advanced Center for Chronic Diseases (ACCDiS), Institute of Biomedical Sciences (ICBM), Facultad de Medicina, Universidad de Chile. (Sponsored by FONDECYT 1110149 (LL), 1130250 (AFGQ); BNI P09-015-F (LL); ACT1111 (AFGQ); FONDAP 15130011 (AFGQ); CONICYT Student Fellowship (FB).)

Reactive astrocytes are a major impediment to axon regeneration after Central Nervous System (CNS) injury. Our laboratory has shown that avb3 integrin and Syndecan-4, both expressed on a cell line model of reactive astrocytes, interact with neuronal Thy-1 to suppress neurite outgrowth. In vivo, avb3 integrin is only expressed under proinflammatory conditions; thus, we proposed that primary astrocytes treated with TNF-a increment avb3 integrin and Syndecan-4 and induce neurite outgrowth inhibition. Levels of avb3 integrin and Syndecan-4 are assessed by W. blotting. To study neurite outgrowth, CAD cells are seeded over a monolayer of TNF-a-treated astrocytes, and neurite extension is induced by serum deprivation. To evaluate participation of avb3 integrin and Syndecan-4, astrocytes are pre-incubated with anti-b3, heparitinase or Syndecan-4 expression is knocked down. We found that TNF-a increased avb3 integrin and Syndecan-4 expression in astrocytes. Reactive astrocytes inhibited neurite outgrowth in CAD cells. In addition, heparitinase treatment and Syndecan-4 silencing attenuated the inhibition of neurite outgrowth. These effects were potentiated when b3 integrin was also blocked. Our results suggest that both, αvβ3-integrin and Syndecan-4, participate in the inhibition of axonal regeneration mediated by reactive astrocytes after CNS injury.

31) Binding of marine toxins to carboxylic acid modified surfaces

Bustos, P[1]., Gaete, D[1].,Pinto, A[2].,Conejeros, P[1].,[1]Centro de Investigación y Gestión de Recursos Naturales, Facultad de Ciencias, Universidad De Valparaíso.[2]Departamento de Ciencia y Tecnología de Polímeros Universidad del País Vasco/ Euskal Herriko Unibertsitatea. (Sponsored by Fondecyt 11110050 Y PIA ACT 1108)

Saxitoxin and Gonyautoxin are de main components of the paralytic shellfish poison from red tides. A methodology for covalently binding the toxins to carboxile modified surfaces is presented here. Both toxins were successfully bound to magnetic beads and saxitoxin was additionally bound to a modified golden surface in order to perform a surface plasmon resonance analisis. Success of binding to magnetic beads was evaluated through a standard immune-based toxin assay yielding maximum binding values with toxin concentrations of 48 ug/mL for saxitoxin and 32 ug/mL for Gonyautoxin.

32) Theoretical study of the affinity between the Protein Kinase PKA and peptide substrates derived from Kemptide using MM/GBSA.

Caballero, J[1]., Mena-Ulecia, K[2].,Vergara-Jaque, A[2].,Poblete, H[2].,Tiznado, W[2].,[1]Centro de Bioinformatica y Simulacion Molecular, Facultad de Ingenieria, Universidad De Talca.[2]Departamento de Ciencias Químicas Universidad Andrés Bello. (Sponsored by Project FONDECYT Regular # 1130141)

We have carried out molecular dynamics (MD) simulations and MM/GBSA free energy calculations on the complex between the protein kinase A (PKA) and the specific peptide substrate Kemptide (LRRASLG). The same calculations were accomplished on other PKA complexes that contain Kemptide derivatives (with mutations of the arginines, and with deletions of N and C-terminal amino acids). We predicted the experimental observed shifts in the free energy changes from the free PKA to PKA-substrate complex when Kemptide structure is modified. The calculated shifts correlate with the experimental shifts of the free energy changes from the free PKA to the transition states determined by the catalytic efficiency (kcat/KM) changes. Our results demonstrate that it is possible to predict the kinetic properties of protein kinases using simple computational methods. As an additional benefit, these methods give detailed molecular information that permit the analysis of the atomic forces that contribute to the affinity between protein kinases and their substrates.

33) Discovery of new TRPV1 activators analogues to capsaicin in a rational framework

Cáceres, J[1]., Sepúlveda, R[1,2].,Navas, C[1].,Latorre, R[3].,González-Nilo, F[1].,[1]Center for Bioinformatics and Integrative Biology (CBIB), Facultad de Ciencias Biológicas, Universidad Andrés Bello.[2]Programa de Doctorado, Facultad de Ciencias Biológicas, Universidad Andrés Bello.[3]Centro Interdisciplinario de Neurociencias de Valparaíso (CINV), Facultad de Ciencias, Universidad De Valparaíso. (Sponsored by CINV Is A Millennium Institute Supported By The Millennium Scientific Initiative Of The Ministerio De Economía, Fomento Y Turismo (09-022-F). This Work Was Supported By FONDECYT 1131003. RV Sepúlveda Thanks CONICYT-Chile For A Doctoral Fellowship.)

The TRPV1 channel is a polymodal non-selective cation channel whose has been proposed as the integrator of diverse noxious stimulus in the pain generation pathway. It is known that can be activated by ligands like capsaicin or by noxious heat (>42°C) but the structural events that the channel undergoes during gating are still unknown. Consequently, the understanding of the structural background of the channel provide exciting opportunities for pharmacological intervention.The aim of this research is to identify novel activators of TRPV1 by taking the characterization of capsaicin as a model for structure-ligand interaction and to unravel the structure-function relationship involved in channel gating via molecular dynamics (MD) simulations. Our early MD studies has been indicated that the capsaicin-dependent activation of the channel involves a shift in the curvature of a key segment, which disrupts the pore domain and molecular docking assays shows the influences ruling the orientation of capsaicin in the binding pocket. Furthermore with a massive molecular docking strategy we have tested a database of 112.935 small ligands selecting a group of 10 molecules by using the binding affinity and the hydrophobicity as selection criteria.

34) Structural and functional analysis of a Kunitz trypsin inhibitor produced in poplars under copper stress

Campos, C., Guerra, F[1].,Reyes, L[2].,Blaudez, D[3].,Gutierrez, A[1].,Ruiz-Lara, S[1].,[1]Instituto de Ciencias Biológicas Universidad de Talca.[2]Escuela de Ingeniería en Bioinformática Universidad de Talca.[3]Faculty of Science and Technology Université de Lorraine, Nancy, France. (Sponsored by FONDECYT 11110214)

Poplars (*Populus* spp) have been proposed as candidate species for phytoremediation of heavy metals. Copper is an essential micronutrient for plants and a common pollutant in some countries. In previous studies, we identified a suite of genes differentially expressed in poplars subjected to copper excess that included some genes encoding members of the Kunitz trypsin inhibitor (KTI) protein family, traditionally associated with the cell defense against herbivores and pathogen attack. However, the relationship of KTIs and heavy metal stress is scarcely known. In order to determine the role of KTIs in the mechanisms of copper tolerance, we focused this study on the structural and functional characterization of the *Populusdeltoides* KTI3 gene (*PdKTI3*). *PdKTI3* was isolated and sequenced, and its protein structure was modeled. The predicted structure showed three putative copper binding domains, suggesting the ability of the protein for chelating this metal. Additionally, expression analyses showed a significant up regulation of *PdKTI3* in both roots and leaves, when hydroponically grown poplars were treated with copper. We also analyzed the sub-cellular localization of PdKTI3 by transient expression in onion cells. The PdKTI3:GFP product was detected all over the cell, without an organelle-specific pattern. The results suggest that PdKTI3 would be a chelating protein involved in the defense mechanisms induced by copper stress, a novel role for the KTIs.

35) Integrative gene network analysis uncovered a relationship between nitrate and root hair development in *Arabidopsis thaliana*.

Canales, J[1]., Gutiérrez, R[1].,[1]Millennium Nucleus Center for Plant Functional Genomics. FONDAP Center for Genome Regulation. Genética Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica De Chile. (This Work Was Funded By FONDECYT Postdoctoral Fellowship (3130315), FONDECYT (1100698), Howard Hughes Medical Institute (Award 55007421), FONDAP Center For Genome Regulation (Grant 1509007), Millennium Nucleus Center (Grant P10-062-F))

Nitrogen (N) is an essential macronutrient for plant growth and development. Plants adapt to changes in N availability partly by changes in global gene expression. Meta- analysis of publicly available root microarray data under contrasting nitrate conditions identified new genes and functions important for adaptive nitrate responses in *Arabidopsis thaliana* roots. Our integrative bioinformatics analysis allowed us to postulate the hypothesis that root hair development is an important developmental process in response to nitrate in Arabidopsis. Root hairs are specialized epidermal cells involved in water and nutrient uptake. In order to test this hypothesis, we performed treatments with nitrate or potassium chloride in hydroponic media of wild-type plants that were previously grown for two weeks in a low nitrogen media. We observed a two-fold increase in root hair density in response to nitrate treatments. However, mutants related to nitrate transport and control of nitrate response have a small increase of root hair number. These results suggest that there is a strong link between nitrogen availability and the development of root hairs and identified regulatory factors that mediate this adaptive response in Arabidopsis roots.

36) *Helicobacter pylori*- induced HIF-1α stabilization and transcriptional activation is linked to virulence factor CagA-dependent activation of phosphatidylinositol 3- kinase (PI3K)-AKT pathway.

Canales, J[1]., Valenzuela, M[1].,Bravo, D[2].,Toledo, H[3].,Quest, A[1].,[1]Laboratorio de Comunicaciones Celulares, Centro de Estudios Moleculares de la Célula (CEMC), Centro de Estudios Avanzados en Enfermedades Crónicas (ACCDiS), Programa de Biologia Celular y Molecular, Instituto de Ciencias Biomédicas (ICBM) , Facultad de Medicina, Universidad De Chile.[2]Laboratorio de Microbiología Oral, Departamento de Patología y Medicina Oral, Facultad de Odontología, Universidad De Chile.[3]Laboratorio de Microbiología Molecular, Programa de Biología Celular y Molecular, Instituto de Ciencias Biomédicas (ICBM), Facultad de Medicina, Universidad De Chile. (Sponsored by FONDECYT 1130250, ACT1111, FONDAP 15130011 (AFGQ), FONDECYT 1120126 (HT),FONDECYT 11110076 (DB), CONICYT Student Fellowship (JC))

Helicobacter pylori (Hp) promotes cancer by activating pathways with carcinogenic potential via the virulence factor CagA. Also, Hp promotes stabilization of the hypoxia inducible factor HIF-1a, which is strongly implicated in tumor progression. However, the virulence factors and cellular mechanisms involved remain unclear. In other models, HIF-1a stabilization is mediated by activation of the PI3K-AKT pathway. Here we determined whether CagA-mediated PI3K/AKT activation is relevant to stabilization and transcriptional activation of HIF-1a induced by Hp. The gastric cell lines MKN45 and AGS were exposed to the Hp strain 26695, Hp strain 84-183 or the isogenic ΔcagA mutant Hp strain. Expression of HIF-1a and Akt phosphorylation were evaluated by western blotting. HIF-1 transcriptional activity was measured by a luciferase reporter assay.Hp induced HIF-1 transcriptional activity was found to depend on the multiplicity of infection. HIF-1a protein levels increased transiently following Hp infection and correlated with Akt phosphorylation. The PI3K inhibitor LY294002 and the Akt inhibitor Akti1/2 reduced Hp-induced HIF-1a protein levels and HIF-1 transcriptional activity. Moreover, Hp strain 84-183, but not the isogenic ΔcagA mutant promoted HIF transcriptional activity. In summary, Hp induces HIF-1a stabilization and activation in a CagA and PI3K-AKT-dependent manner.

37) Expression analysis of the Toll-like receptors involved in viral response of healthy and diseased salmon with IPN virus

Cardenas, T[1]., Figueroa, J[2,1].,Haussmann, D[2,1].,[1]Instituto de Bioquímica y Microbiologia, Ciencias, Universidad Austral De Chile.[2]Interdisciplinary center for Aquaculture Research , (INCAR), Centro FONDAP.

The Infectious Pancreatic Necrosis (IPN) is a disease that mainly affects Atlantic salmon (*Salmo salar*), causing large economic losses mainly in juvenile state and pre-smolt fish. This disease is caused by IPN virus, characterized for being a double-stranded RNA virus.

On the other hand in innate immune response, main receptors responsible for recognizing pathogens, such as virus, are toll-like Receptors (TLRs). A group of them recognize virus associated patterns. TLRs are transmembrane proteins, that when bound to its ligand (PAMs: pamps pathogen-associated molecular patterns), can cause inflammatory responses and cytokine production.

In this work, cDNA from head kidney of healthy fish, asymptomatic and symptomatic (infected with IPNv) was used to quantify TLRs expression by RT-qPCR. Expression of TLRs that recognize as ligand double-stranded RNA (TLR3, 7, 9, 13 and 22) were analyzed. In parallel we study the protein expression level of TLR22 by immunofluorescence and Western blot in head kidney and spleen. Preliminary results indicate that exist variation in expression between healthy and infected individuals. With respect to the results obtained with TLR22, this receptor shows increased expression in symptomatic fish compared to the healthy and asymptomatic salmons.

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38) PAR-3 and Syndecan-4 are involved in astrocyte adhesion induced by neuronal Thy-1

Cárdenas, A[2]., Kong, M[1].,Quest, A[1].,Leyton, L[2].,[1]Cellular Communication Laboratory, Advanced Center for Chronic Diseases (ACCDiS) , Faculty of Medicine, Universidad De Chile.[2]Cellular Communication Laboratory, Advanced Center for Chronic Diseases (ACCDiS), Biomedical Neuroscience Institute (BNI), Institute of Biomedical Sciences (ICBM) , Faculty of Medicine, University De Chile. (Sponsored by FONDECYT 3140471 (AC), ICM-P09-015F, FONDECYT 1110149 (LL), FONDECYT 1130250, ACT111, CONICYT-FONDAP 15130011 (AFGQ).)

Introduction: We have shown previously that astrocyte adhesion is induced by the interaction of the neuronal protein Thy-1 with avb3 integrin and Syndecan-4 in astrocytes. However, the signaling mechanisms triggered downstream of these astrocyte receptors remain unknown. PAR-3 is an astrocyte protein implicated in migration, but its function in astrocyte adhesion remains to be determined. Here, we evaluated the participation of both Syndecan-4 and PAR-3 in astrocyte adhesion induced by Thy-1.

Material and methods: Rat DITNC-1 astrocytes were transfected with siRNA against PAR-3, Syndecan-4 or siRNA control, treated with Thy-1-Fc or TRAIL-R2-Fc as a control and then assayed for wound-healing and focal adhesion formation. For focal adhesion analysis the cells were stained with anti-vinculin. Focal adhesion number and morphology were evaluated by confocal imaging.

Results and discussion: Our results show in DITNC-1 cells transfected with siRNA for PAR-3 or Syndecan-4 and stimulated with Thy-1 that wound-closure was decreased compared with siRNA control-transfected cells. For cells transfected with PAR-3 or Syndecan-4 siRNA larger focal adhesions than control cells were observed, implicating PAR-3 and Syndecan-4 in astrocyte adhesion induced by Thy-1. Whether these proteins regulate focal adhesion disassembly will be addressed in future studies.

39) Metabolic shift induced by PDGF-BB involves mitochondrial fragmentation in VSMCs

Cartes-Saavedra, B[1]., Mondaca-Ruff, D[1].,Norambuena-Soto, I[1].,Vidal-Peña, G[1].,Morales, PE[1].,García-Miguel, M[1].,Mellado, R[2].,Lavandero, S[1,3].,Chiong, M[1].,[1]ACCDiS, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad De Chile.[2]Departamento de Farmacia, Facultad de Química, Pontificia Universidad Católica De Chile.[3]ACCDiS, Facultad de Medicina, Universidad De Chile. (Sponsored by Fondecyt 1140329, FONDAP 15130011, Anillo ACT1111. D.M-R. Holds A Conicyt Fellowship)

Vascular Smooth Muscle Cells (VSMCs) are important in the regulation of vascular tone and blood pressure. Mature VSMCs have a contractile phenotype with very low proliferation rate. Platelet-derived growth factor BB (PDGF-BB) is a key factor in the development of vascular diseases such as stenosis and atherosclerosis. In these pathologies, VSMCs change their contractile to a secretory phenotype. We explored whether mitochondrial metabolism and dynamics are affected during PDGF-BB-induced phenotypic switching. Smooth muscle A7r5 cells were treated with PDGF-BB (10 nM) for 0-48 h. Mitochondrial dynamics was assessed by confocal microscopy and mitofusin-2 protein level by Western blotting. Metabolism was assessed by ATP levels and mitochondrial potential (Δψm). Our results show that PDGF-BB-induced mitochondrial fragmentation at 3 h, associated with a decrease of Δψm. A decrease in mitofusin-2 protein level was detected at 24 h. No significant decrease of total ATP level was observed. These changes precede the appearance of synthetic phenotype characterized by an increase of collagen I level and a decrease in myosin heavy chain level. Changes in metabolism and mitochondrial dynamics could be an important hallmark in the phenotypic switching and development of vascular diseases.

40) Polymorphisms in the 11beta-Hydroxysteroid dehydrogenase type-2 promoter decrease the human HSD11B2 expression *in vivo*

Carvajal, C[1]., Lizama, J[1].,Reyes, M[1].,Valdivia, C[1].,Campino, M[1].,Vecchiola, A[1].,Lagos, C[1].,Allende, F[2].,Solari, S[2].,Fardella, C[1].,[1]Endocrinology, Medicine, Pontificia Universidad Católica De Chile.[2]Laboratorios Clinicos, Medicine, Pontificia Universidad Católica De Chile. (Funded By FONDECYT 1130427, SOCHED 2012-04, FONDEF IDeA CA12i10150, Millennium Institute Of Immunology And Immunotherapy IMII P09/016-F (ICM) And CORFO 13CTI-21526-P1.)

The expression and activity of 11beta-hydroxysteroid-dehydrogenase type 2 (11BHSD2) gene can be affected by mutations, polymorphisms (SNP) and epigenetic modifications. SNPs in HSD11B2 promoter may impair its normal expression and furthermore the activity.

Aim: To study the presence of SNPs in HSD11B2 promoter and its impact in HSD11B2 RNA expression and biochemical parameters in humans.

Subjects and Methods: We studied 105 subjects. We measured plasma renin activity (PRA), serum aldosterone and urinary F and E. We obtained RNA and DNA from PBMC and amplified the HSD11B2 proximal promoter (NG_016549). To identify polymorphisms (SNP) we did PCR-HRM and sequencing. We analyzed eleven SNPs in HSD11B2 proximal promoter, and the HSD11B2/U6 expression in PBMC by RT-qPCR.

Results: We detected heterozygous alterations in 2/11 SNPs of HSD11B2: rs45598932 (G-209A) and rs56057545 (G-126A). We identified these SNPs in 9/105 subjects: G-209A (4/105, 3.8%) and G-126A (5/105, 4.7%). The HSD11B2 RNA expression was lower in subjects carrying the SNPs than native (0.37 vs. 1.26 AU, p 0.04). In pediatric subjects carrying the SNPs, we found a trend to higher urinary F/E ratio (0.41vs. 0.30, p 0.08).

Conclusions: HSD11B2 promoter polymorphisms, G-209A and G-126A, showed a minor allele frequency (MAF) of 3.8% and 4.7%, similar to NCBI (2.4% and 4.8%). Subjects carrying these SNPs showed a lower mRNA expression of HSD11B2 promoter than those with native promoter, which could affect the activity of 11BHSD2 *in vivo*, especially in pediatric subjects.

41) Identification and partial biochemical characterization of a phytase active at low temperatures

Castillo, B[1]., Álvarez, A[1].,Pozo, P[2].,Reyes, A[1].,Costa, M[2]., [1]Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral De Chile.[2]Instituto de Ciencia y Tecnología de los Alimentos, Facultad de Ciencias Agrarias, Universidad Austral De Chile. (Sponsored by FONDEF CA1210022, DID-UACh S-2012-46)

As a consequence of the decreasing availability of animal protein, fish feedstuff as been increasingly supplemented with protein from vegetal sources. However, the presence of high amounts of phytic acid (PA) in the plant feedstuff has brought economic and environmental problems. As PA is not bio-assimilated, it causes malnutrition in fishes and promotes the eutrophication of the environment when excreted, for instance causing algal blooms. Attempts to supplement plant feedstuff with phytase, an enzyme that hydrolyzes PA to inorganic phosphorus plus inositol-phosphates, to make phytic-phosphate assimilable by fishes failed since commercially available enzymes works best at temperatures above than those found in live fish and breeding areas. To identify a phytase active at low temperature (14ºC), suitable as a food supplement for fishes, a library of microorganisms was screened to select a fungus that grows at low temperature and with PA as the sole source of phosphorus. In solid and liquid media this microorganism, identified by ITS1-5.8S-ITS2 sequencing, releases an extracellular phytase with high activity at 14°C. The enzyme has a molecular mass >30 kDa and exhibit a K_M and V_{max} value for PA of 30 µM and 80 µM min^{-1}, respectively. Calcium has a stimulatory effect on phytase activity at low levels (until 0,5 mM), and an inhibitory effect at higher concentrations. This phytase with high activity at low temperatures has great potential for feed applications, especially for aquaculture.

42) Engineering of Dengue Fever NS1-based epitopes

Coelho, D[1]., Rusu, V[2].,Junior, S[1].,Lins, R[1].,[1]Departamento de Química Fundamental Universidade Federal de Pernambuco.[2]Department of Physical Chemistry ETH-Hönggerberg. (This Work Is Supported By FACEPE, CNPq, CAPES, Nanobiotec-BR, INCT-IMANI, LAVITE-CPqAM. Partial Computer Allocation Was Granted By Swiss Supercomputing Centre (CSCS), ACLF At Argonne National Laboratory And HPC2N At Umeå University, Sweden.)

Dengue fever is a viral disease that can result into a systemic syndrome and no prophylactic treatment or vaccine is available. The dengue virus (DENV) presents itself in 5 serotypes, namely DENV-1, -2, -3, -4 and -5. NS1 is a non-structural 352-residues long protein encoded by DENV genome that can be found in the blood of infected people, acting as an antigen and activating the complement system. The region between the 221 to 266 amino acids of NS1 has been identified as containing an epitope. This region was capable to elicit anti-NS1 antibodies that had cross-reaction with DENV-1, -2 and -3. The aim of the current work is to engineer chimerical proteins, containing the putative NS1 epitopes sequences, as potential candidates as vaccine and/or diagnostic antigens. The identified sequences were inserted into four scaffolds well known by their thermal and environmental stabilities: Top7, Thioredoxin-A, GB1 and SSO7D. The protocol consisted in selecting short sequences of the NS1 protein and verifying in what regions of the scaffold it can be accommodated. The stability of over 300 chimeras was initially estimated by *de novo* techniques using the Rosetta 3.5 software. The most energetically stable mutants were selected and had their structural and dynamical stabilities assessed by molecular dynamics simulations using the Gromacs 4.6.5 package. Our results revealed a few promising epitope-carrying chimeras that can be heterologous expressed and immunogenically tested.

43)

44) The WNT1 LIGAND/FRIZZLED 3 receptor system plays a regulatory role in the achivement of the in vitro capacitation and subsequent *in vitro* acrosome exocytosis of porcine spermatozoa

Covarrubias, A[1]., Cereceda, K[1].,Vander Stelt, K[1].,López, C[1].,Montes De Oca, M[1].,Rodríguez-Gil , J[2].,Concha, I[1].,[1]Bioquimica, Ciencias, Universidad Austral De Chile.[2]Unidad de reproducción animal, de Veterinaria, Universidad Autonoma de Barcelona. (Sponsored by FONDECYT 1110508 (IC), FONDECYT 1141033 (JCS), AGL2008-01792/GAN (JER-G), DID-UACh 1330-32-09 (AC), AC: Beca CONICYT, MECESUP AUS 0704.)

The aim of this work was to determine the existence of a functional WNT/β-catenin signaling pathway in boar spermatozoa, which would be linked with the already well known GSK-3 signaling pathway. This was firstly confirmed by detecting the presence of the specific Frizzled 3 receptor in these cells. Furthermore, this signaling pathway was activated in boar sperm subjected to “in vitro” capacitation (IVC) and subsequent progesterone-induced “in vitro” acrosome exocytosis (IVAE) by incubating cells with separate concentrations of the WNT/β-catenin signaling pathwayspecific effector Wnt1 ligand. Incubation with the Wnt1 ligand decrease the percentage of viability during the IVC and IVAE. This was concomitant with a time-dependent increase of sperm with altered membrane fluidity (Merocyanine-540). On the contrary, the Wnt1 ligand did not modify the total motility during the IVC. However, the Wnt1 ligand induced an increase in the motility patterns of sperm subjected to IVAE. This action was linked to a decrease in the percentage of cells with high mitochondrial membrane potential and an increase in the percentage of cells with high intracellular Ca^{2+} content. In conclusion, our results suggest that the Wnt ligands-modulated WNT/β-catenin signaling pathway is playing a relevant role in the modulation of both IVC and subsequent, progesterone-induced IVAE. Furthermore, our results seem also to indicate that the transduction pathways by which the Wnt1 ligand acts on IVC and IVAE are different and, in case of IVC, independent of the GSK-3 activity.

45) Molecular cloning and recombinant expression of low molecular weight peptide present in the venom of *Loxosceles laeta* (corner spider)

CRUZ, V[1]., Ordenes, K[1].,Catalán, A[1].,Araya, J[1].,Orrego, P[2].,[1]DEPARTAMENTO DE TECNOLOGIA MEDICA, FACULTAD CIENCIAS DE LA SALUD, Universidad De Antofagasta.[2]Biomédico, Facultad de Ciencias de la Salud, Universidad De Antofagasta. (Sponsored by Jorge González Cortes)

Poisonous spiders are the most abundant arthropod predators on earth. The genus *Loxosceles* are found worldwide. *L. laeta* is endemic of South America.

The spider venom is a complex mixture of labile protein toxins and peptides, proteins containing hydrolases, lipases, peptidases, collagenases, alkaline phosphatase, 5-ribonucleotides, phospho hydrolases, proteases, hyaluronidases, phosphatases and phospholipases A2 acid, metalloproteases, serine proteases and insecticidal toxins. Several approaches have been directed towards to found new molecules with insecticide activity, affecting only the pest and the surrounding flora and fauna, among the organisms studied are plants bacterias, animals, arthropods; highlighting various species of spiders, scorpions, snakes, due to the large number of toxins present in the venoms. Because of this we are motivated to the search of genes on the cDNA of the glandular venomous tissue of *L. laeta* that code for peptides with potential insecticidal activity. Using molecular strategies we cloned, heterologously expressed and characterized the functionality of a peptide of low molecular mass, called LlTx3 who has an estimated molecular weight of 9.3 kDa and a pI of 8.04. Insectidal activity assays were performed using as model of larval mealworm, *Tenebrio molitor*, showing a dose-dependent larvicidal activity. These results report a new insecticide peptide present in the venom of *L. laeta* with a potential action more safer and wider.

Proyecto FONDEF IDeA CA12I10298

46) Functional characterization of a cysteine-less isoform of the human GLUT2 hexose transporter

Cuevas, A[1]., Arce, R[1].,Salas, M[1].,Reyes, A[1].,[1]Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral De Chile. (Sponsored by FONDECYT 1130386, FONDEF D11I1131)

The GLUT2 carrier is the major glucose transporter isoform expressed in hepatocytes, insulin-secreting pancreatic beta cells, and absorptive epithelial cells of the intestinal mucosa and kidney. Due to it functions as a low affinity, high-turnover transport system it is thought to act as a glucose-sensing apparatus that plays a role in blood glucose homeostasis, by responding to changes in blood glucose concentration. Cysteine scanning mutagenesis in conjunction with SH group chemical modification has proved to be a powerful strategy for investigating structure and function of integral membrane proteins. Therefore, a cysteine-less isoform of the GLUT2 carrier (C-less GLUT2) was designed and expressed in Xenopus oocytes. This C-less GLUT2 was refractory to MTSET, an membrane-impermeant alkilating reagent of thiol groups that obliterates the activity of native GLUT2. The C-less GLUT2 transport methylglucose and deoxyglucose and transport is competed by fructose, sorbitol, methyl α-D-glucopyranoside, galactose and ribose. Besides, C-less GLUT2 activity was hampered by cytochalasin B, quercetin and resveratrol. The properties of C-less GLUT2 are similar to those of the native GLUT2, which suggests that this C-less isoform is a suitable framework to perform structure-function analysis by Cys-scanning mutagenesis.

47) Development and Implementation of a GROMOS-Based Force Field for Atomistic Simulations of Peptoids

Cunha, K[1]., Coelho, D[1].,Lins, R[1].,[1]DQF , Chemistry , Universidade Federal de Pernambuco. (Sponsored by CNPq, Capes And FACEPE)

Peptoids are polymers comprised of N-substituted glycine monomers. These biomimetics display a wide structural and functional diversity. The main advantages of peptoids, over peptides, are their thermal and environmental stability, proteolysis resistance and simple synthesis due to the lack of a chiral center. Despite the variety of biological applications that peptoids can be used for, relatively few efforts have been done to characterize their structures and how they correlate with biological function in a predictive manner. Molecular dynamics simulation has been a powerful tool in the investigation of structural features of proteins and therefore could also be used for the study of peptides. However, the lack of parameters (force field) for peptoids is currently the major limiting factor. To overcome this limitation, we have developed a peptoid library, namely Pitt-2, for the GROMOS force field (parameter set 54A7). This library consists of 10 peptoid residues that can be combined to generate millions of different sequences and has been used for chronological diagnosis of HIV-1 patients. Force field validation was carried out by comparing the results from the classical simulations with available QM and experimental data. Moreover, solvation effects were evaluated and shown to play a role in the free energy landscape even for apolar peptoids. The parameters, compatible with the remaining of the GROMOS force field, were implemented into the GROMACS simulation package.

48) *ERF115*, a gene involved in the tolerance to salt stress in *Arabidopsis thaliana*

Del Rio, V[1]., León, L[1].,Salinas, P[1].,Holuigue, M[1].,[1]Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile. (Sponsored by Supported By FONDECYT 1141202 And Millennium Nucleus For Plant Functional Genomics P10-062-F.)

Abiotic stresses can severely impair plant growth and development, being the salinity one of the main problems for agricultural productivity. We identified *ERF115*, a gene coding for a putative transcription factor that is induced in roots by salt treatments. Functional analysis of a homozygous mutant line showed to be less tolerant than the WT plants to salinity stress. We have complemented *erf115* mutant plants with constructs either to overexpress this gene or express it controlled by its own promoter. Likewise we were able to complement a germination phenotype in salt. In these lines we also evaluated the expression of genes which were previously determined by microarrays experiments as affected in the mutant line. All together, and considering that ERF115 localizes in the nucleus and is able to activate the transcription in a yeast assay, we suggest that ERF115 plays an important role as a transcription factor in the tolerance to salinity stress, regulating the expression of genes involved in this plant stress response.

49) Insights into the role of the CRE-1 transcription factor in the circadian regulation of cellulose-metabolism in the fungus *Neurospora crassa*

Díaz, R[1]., Larrondo, L[1].,[1]Millennium Nucleus for Fungal Integrative and Synthetic Biology and Depto. Genética Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica De Chile. (Sponsored by FONDECYT 1131030 To LFL. RDC Is Supported By A CONICYT Fellowship For Doctoral Studies And By MN-FISB NC120043).

Circadian clocks are autonomous molecular-timers composed of interconnected transcriptional/transcriptional feedback loops. They are thought to confer a selective advantage to organisms by enabling processes to occur at appropriate times of the day. In the model organism *Neurospora crassa*, ~20% of its genes are under circadian control and interestingly, many of them are related to diverse metabolic processes such as cellulose degradation. However, the pathways involved in relaying the time-of-day information to the expression of the cellulolytic genes, remains unclear. We are interested in elucidating the transcriptional mechanisms interconnecting circadian and metabolic processes, particularly regarding organismal fitness. Thus, we are analyzing carbon catabolite repression (CCR) and cellulolytic capabilities in a circadian context, through the evaluation of the circadian role of CRE-1, a transcription factor known to be key in modulating catabolism, and herein shown to be involved in the aforementioned processes. We found evidence that suggests that this transcription factor acts as a link between the circadian and metabolic pathways. These data provide insights on how the circadian clock is influencing Neurospora physiology, potentially impacting a biotechnological relevant process like biomass conversion.

50) Testing the hydration of the shaker-K channel sensor domain with sugar

Diaz-Franulic, I[1]., Naranjo, D[1].,[1]Neurociencias, Ciencias, Universidad De Valparaíso, CINV. (Sponsored by ID-F Is A MECESUP Fellow. We Thank To Iniciativa Cientifica Milenio (P09-022-F) And Fondecyt 1120819).

Voltage gated K-channels subunits are formed by two well defined domains: the Pore Domain (PD), which is responsible for the K^+ ions conduction process, and the Voltage Sensor Domain (VSD), which sense the transmembrane potential due to the presence of several charged moieties. VSD moves upon activation such that ~4 net positive charges translocate across the membrane. The molecular details of such movement have been subject of intense controversy. We asked if part of the charges are hydrated at the resting and activated states, and if they change their hydration status during voltage activation. We measured the gating currents of a constitutively closed Shaker-V478W in macro patches of *Xenopus* oocytes in the presence of internal, external, or symmetric 2M Sucrose to reduce the water availability for eventual VSD hydration. Our results are consistent with the idea that some charged residues are hydrated when exposed to the cytosol at resting, dehydrate before translocation and rehydrate externally in the activated conformation. These suggest that water plays an important role stabilizing charged moieties in both, the resting and active conformation, revealing a novel role for water in the voltage sensing process.

51) Apoptotic cell bodies from tumor cells as an alternative immunotherapy against breast cancer

Escrig, D[1]., Mena, J[1].,Perez, D[1].,Barrientos, C[1].,Faundez, A[1].,Lopez, X[1].,Rojas, J[2].,Acuña-Castillo, C[1].,[1]Centro de Biotecnología Acuícola, Facultad de Química y Biología, Universidad de Santiago de Chile.[2]Patología Centro Asistencias Barros Luco. (Sponsored by FONDECYT 1110734).

We development a prophylactic immunotherapy based on “cell bodies (CB)” plus Polymyxin B and ATP in murine B16 melanoma model. Now we evaluated this treatment in breast cancer model, as a healing immunotherapy. Also we tested modified the “cell bodies” with Dinitrochlorobenzene (DNCB) a hapten capable to increase the immunity response. In this work, Balb/c mice were challenged with 4T1mOVA breast cancer cell line, the tumor growth was determined daily until a growth of 0.5 cm^3. The vaccine was applied on day 7, 14 and 21 after the challenge with the tumor cells. In the vaccine with modified CB, mice were sensitized with DNCB a week before the challenged. The animals were sacrificed and CD8+, CD4+ and Treg lymphocytes levels were measured in spleen and tumor infiltrates by flow cytometry. All treatments delay the maximal tumor growth compared with control animals. However, splenic $CD4^+$, $CD8^+$ and Treg lymphocytes didn’t show mayor differences between experimental and control groups. But the treatment with CB plus ATP and PMB was capable to increases the CD4+ lymphocytes infiltrating in tumor. In conclusion, treatments with CB are a potential immunotherapy capable of delay the tumor growth and increase lymphocytes infiltrating in tumor. New experiment with a vaccine that combines these two treatments will be done.

52) SIZ1 in the regulation of salicylic acid biosynthesis in *Arabidopsis*.

Farias, D[1]., Salinas, P[1].,Holuigue, M[1].,[1]Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile. (Supported By FONDECYT (1141202) And NM-GFP (P10-062-F)).

SUMOylation is a post translational modification that involves covalent attachment of a SUMO (Small Ubiquitin-like Modifier) peptide to target proteins affecting their functions. Protein SUMOylation involves three enzymes: E1, E2 and E3. SIZ1 is an E3 sumoligase enzyme from *Arabidopsis* involved in various stress responses. *siz1* mutant plants show constitutive accumulation of salicylic acid (SA), a plant hormone involved in stress responses. Accumulation of SA in *siz1* plant is correlated with an increased expression of genes involved inSA biosynthesis, such as *ICS1, EDS1, PAD4, CBP60g*, which suggests involvement of SIZ1 in a negative control mechanism of SA biosynthesis. On the other hand, EIN3 is a transcripcional factor involved in the ethylene signaling and in the repression of SA biosynthesis. EIN3 was described in a massive screening of SUMOylation targets, but the function of this modification in its activity remains unclear. We propose that the expression of one or more genes involved in SA biosynthesis is repressed under basal conditions, in a mechanism depending of EIN3 SUMOylation. To test this hypothesis, we evaluated in an *in vivo* SUMOylation assay if SUMOylation of EIN3 is SIZ1-dependent. Then we identified genes involved in SA biosynthesis that are deregulated directly by the absence of SIZ1 activity in *siz1* plant that express NahG gene. This analysis will allow us to identify potential targets regulated by SIZ1-mediated SUMOylation of EIN3.

53) The role of glucocorticoid receptor-b in autophagy

Farías, M[1,2]., Bernal, I[1,2].,Paredes, F[1].,Diaz, A[1].,Cartes, B[1,2].,Mellado, R[2].,Troncoso, R[1]., [1]Advanced Center for Chronic Diseases (ACCDiS), Facultad de Ciencias Quimicas y Farmaceuticas, Universidad De Chile.[2]Departamento de Farmacia, Facultad de Quimica, Pontificia Universidad Católica De Chile. (Sponsored by FONDECYT 11130285 (RT), FONDAP 15130011 (RT)).

Glucocorticoids (GCs) are steroids hormones that regulate growth, metabolism, autophagy, development and immune functions and play a pivotal role in preserving basal and stress-related homeostasis. The effects of glucocorticoids are mediated by the GC receptor (GR). GR is expressed as two alternately spliced C-terminal isoforms α and β. GR-β does not bind GCs, but the synthetic drug RU486 is able to bind and modulate its transcriptional activity. The aim of this work was to study the role of GR-β in the regulation of autophagy in HeLa cells. To evaluate the role of GR-β on autophagy cells were treated with the drug RU486 (1 μM) or with the overexpression of the GR-β. Autophagy was assessed by conversion of LC3-I to LC3-II and levels of p62. Autophagy fluxwas determinedin the presence of the vacuolar ATPase inhibitor,bafilomycin.Our results show that the drug RU486 stimulated the conversion of LC3-I to LC3-II without affecting p62 levels. RU486 also stimulated autophagy flux. Moreover, the overexpression of GR-β also promotes autophagy induction. Our data suggest that the drug RU486 and the overexpression of GR-β activates autophagy.

54) Inflammation is involved in maternal obesity-induced ER stress in human umbilical vein endothelial cells

Farías-Rojas, M[2].,Villalobos-Labra, R[1,2].,Westermeier, F[1,2,3].,Kusanovic, J[1].,Poblete, J[1].,Mardones, F[4].,Sobrevia, L[2].,-Farías-Jofré, M[2].,[1]Division of Obstetrics and Gynaecology, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile.[2]Cellular and Molecular Physiology Laboratory (CMPL), Medical Research Centre (CIM), Faculty of Medicine, Pontificia Universidad Católica De Chile.[3]Advanced Center for Chronic Diseases (ACCDiS), Faculty of Chemical & Pharmaceutical Sciences, Universidad de Chile.[4]Division of Public Health, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica De Chile. (Sponsored by FONDECYT (1121145, 1110977, 1090594), CONICYT (ACT-73 PIA)).

Maternal obesity (MO) is associated with fetal programming of adverse cardio-metabolic outcome in the offspring. Endoplasmic reticulum (ER) stress has been implicated in obesity-dependent complications. There is evidence showing that inflammation induces ER stress, however, the link between MO, inflammation and ER stress is not clear. Here we study the potential role of inflammatory cytokines in detection of ER stress markers in human neonatal endothelial cells from pregnancies with MO. Human umbilical vein endothelial cells (HUVEC) were isolated from normal (HUVEC-N) or MO (HUVEC-OB) pregnancies attending the obstetrics service at the Clinical Hospital of Pontificia Universidad Católica de Chile. We evaluated the effect of TNFα and/or IL10 (1 ng/mL and 50 ng/mL, respectively) on phosphorylated and total protein levels of eIF2α through Western blot. HUVEC-OB showed an increase in phosphorylation of eIF2α compared to HUVEC-N, which was reduced by incubation with IL-10 or IL-10 plus TNFα together. TNFα by itself did not induce changes on eIF2α phosphorylation levels. In conclusion, IL-10 is capable to reduce the MO-dependent activation of the ER stress marker eIF2α in HUVEC. It suggests a potential way to normalize the ER physiology in neonatal vascular cells from pregnancies affected by MO, using anti-inflammatory tools.

55) Heterologous expression and characterization of a β-xylosidase from *Penicillium purpurogenum.*

Faundez, C[1]., Perez-Lara, R[1].,Mardones, P[1].,Eyzaguirre, J[1].,[1]Ciencias Biologicas, Ciencias Biologicas, Universidad Andrés Bello. (Sponsored by FONDECYT 110084 And 1130180, UNAB DI-478-14/R).

Xylan, a component of plant cell walls, is composed of a backbone of β-1,4-linked xylopyranosyl units with a number of substituents. The complete degradation of xylan requires the action of several enzymes, among them β-xylosidase. The fungus *Penicillium purpurogenum* secretes a number of enzymes participating in the degradation of xylan. In this study, a ß-xylosidase from this fungus was expressed in *Pichia pastoris*, and characterized. This enzyme (XylII) is a member of glycosyl hydrolase family 3 with a theoretical molecular mass of 84118.81 and isoelectric point of 5.07, and a signal peptide of 20 residues. The highest identity with a characterized enzyme, is with a β-xylosidase from *Aspergillus aculeatus* (73%). The optimal activity of XylII was at pH 2.0 and 28°C. The enzyme is most stable at pH 2.0 and conserves 50% of activity at 42°C (after 1h incubation). The kinetic parameters for p-nitrophenyl-β-D-xylopyranoside are: Km 0.53 mM, kcat $1*10^7$ s^{-1} and kcat/Km $1.9*10^7$ M^{-1} s^{-1}. The enzyme is about 10% active on p-nitrophenyl-α-L-arabinofuranoside. XylII exhibits a high hydrolytic activity on xylooligosaccharides, and catalyzes hydrolysis of beech and birch glucuronoxylan. This β-xylosidase has a potential use, together with other hemicellulases and cellulases, in processes that hydrolyze lignocellulosic biomass to monosaccharides for fermentation to biofuels and conversion to other value-added products.

56) Effect of cell bodies from 4T1 cells expressing viral fusion proteins on the maturation of dendritic cells

Faundez, A[1]., Barrientos , C[1].,Montoya, M[1].,Cortez, M[1].,Aranda, M[2].,Acuña-Castillo, C[1].,[1]Centro de Biotecnología Acuícola, Facultad de Química y Biología, Universidad de Santiago de Chile.[2]Facultad de Química y Biología Universidad De Santiago De Chile. (Sponsored by FONDECYT 1110734)

Immunogenic cell death (ICD) and cross priming appears to be crucial events for the induction of an effective immune response against cancer. Previously we determined that allogenic cell bodies induce maturation of bone marrow dendritic cells (BMDCs) derived form b6, and the expression of fusogenic viral proteins improves the maturation.

Our aim in this work was determine whether using singenic CBs are able to induce DCs maturation. To prove this it was generated a primary culture of DCs precursors from bone marrow that was stimulated with the cytokine GM-CSF and it was challenged with the different fusogenic CBs. Subsequently it was analyzed the phenotype of the DCs by the presence of maturation markers (CD40, CD86, MHC-I and MHC-II) by flow cytometry.

We demonstrated that singenic cell bodies (CBs), induce the maturation of bone marrow dendritic cells (BMDCs) Balb/c mice, measure an increase on CD40, CD86, MHC-I and MHC-II expression levels. On the other hands, preliminary results obtained from DCs maturation challenged with CBs generated form cells stable expressing human respiratory syncytial virus (hRSV), Murray Valley encephalitis virus (MVEV) or Infectious salmon anemia virus (ISAv) fusogenic viral proteins, appears do not improve the DCs maturation.

57) Polycystin-1 is necessary for cardiomyocyte hypertrophy stimulated by IGF 1

Fernández, C[1]., Torrealba, N[1].,Pedrozo , Z[1].,Lavandero, S[1,2].,[1]Advanced Center for Chronic Diseases (ACCDiS) and Center for Molecular Studies of the Cell (CEMC), Faculty of Chemical and Pharmaceutical Sciences & Faculty of Medicine, Universidad De Chile, Santiago.[2]Department of Internal Medicine, Southwestern Medical Center, University of Texas, Dallas. (Supported By FONDAP 15130011 (SL, ZP), Anillo ACT1111 (SL), U-Inicia (ZP) And CONICYT 24121238 (CF). CF And NT Holds CONICYT Fellowship.)

Cardiac hypertrophy is an early cellular process triggered by chronic biomechanical stress and growth factors such as insulin-like growth factor 1 (IGF-1). Until now the molecular mechanisms involved in the development of cardiac hypertrophy remains uncertain. Polycystin-1 (PC1) is a plasma membrane protein found in different cell types, including cardiomyocytes. In kidney cells, PC-1 acts a mechanosensor and our lab has recently proposed a similar role for PC1 in mechanically stressed cardiomyocytes. In the present work, we study whether PC1 also mediates IGF-1-dependent cardiac hypertrophy.To this end, cultured cardiac myocytes were treated with IGF-1 to trigger hypertrophy. IGF-1 increases β-myosin heavy chain (β-MHC) protein levels and morphometric parameters such as cell perimeter, area and sarcomerization degree. In cardiomyocyte knock down for PC1 with a specific siRNA, the increases in all these parameters were attenuated. Both the phosphorylation of IGF-1 receptor and Akt after IGF-1 stimulation were decreased in PC-1 knock down cardiomyocytes without changes in IGF-1 receptor levels. Collectively, these results show PC1 mediates IGF-1-induced cardiomyocyte hypertrophy.

58) Expression analisis of *gmgt* and *gmmt* genes involved in the structural modification of acemannnan in *Aloe barbadensis* Miller during water stress conditions

Fernández, J[1]., Salinas, C[2].,Cardemil , L[2].,[1]Biología, Química y Biología, Universidad De Santiago De Chile.[2]Laboratorio de Biología Molecular Vegetal, Facultad de Ciencias, Universidad De Chile.

Aloe barbadensis Miller (Aloe vera) is a succulent plant physiologically adapted to arid environments, hence it possess mechanisms that allow it to survive to low water conditions. One of the plant responses to drought that has been studied is the structural modification of the major leaf gel polysaccharide, acemannan. Previous structural analyzes of acemannan from water restricted plants has shown an increase of galactose branches. We hypothesized that the expression of the genes encoding for the enzymes 6-glucomannan-galactosyltransferase, gmgt, and glucomannan 4-beta-mannosyltransferase, gmmt, responsible respectively for the formation of galactose branches and the glucomannan backbone could increase when Aloe plants are subjected to drought. The effects of ABA concentrations in the expression levels of these genes are also contemplated.

Our results indicated that an increase of galactose branches in acemannan varies depending of the stage of plant development, and these changes in the polysaccharide structure can be associated as an adaptation of Aloe to drought. An increased expression level of *gmgt* indicates an early response of the plant to water stress and isn't dependent of endogenous ABA levels. The sequence of gmmt indicated that the amplified region contains conserved domains and the active site characteristic of the glycosyl transferase protein family. BLAST analysis of *gmmt* sequences suggests a high similarity to CslA9 protein.

Acknowledgment: FONDECYT 1130025

59) High glucose modulates the perfil of methilation global in the genomic DNA in human endothelial progenitor cells

Fernández, P[1,2]., Fritz, O[2].,Aguayo, C[3].,Jara, C[3].,Gutierrez, S[1].,[1]Molecular Biology, Biological Sciences, University of Concepción.[2]Medical Technology, Medical Science, University San Sebastián.[3]Clinical Biochemistry and Inmunology, Faculty of Pharmacy, University of Concepción. (Sponsored by DIUSS 2012-0004-I Project, University San Sebastián.)

Endothelial Progenitor Cells (hEPC) plays important functions in postnatal vasculogenesis, specifically in the repair and angiogenesis modulation of damaged tissue. In addition, it has been reported that high glucose conditions (HGC) modifies the behavior of hEPC. In fact, hEPC isolated from diabetic patients have reduced its capacity for migration, adhesion and proliferation compared to control cells, however the molecular mechanisms underlying these alterations mechanism are still unknown. Interestingly it has been observed an altered DNA methylation profile in the pancreatic islets of diabetic patients with differentes pathophysiological significances. Therefore, we hypothesize that the disruption in the adhesion of hEPC under HGC is associated with changes in the DNA methylation in genes involved with vasculogenesis. To test this hypothesis we performed cell culture with HGC. Our results shown that HGC can modify the global DNA methylation status in the hEPC compared with the methylation pattern found in control cells (normal glucose conditions). In addition this conditions not altered the cellular immunophenotype and generates a reduction in the adhesion of hEPC to fibronectin matrix. These results could help to explain the alteration in vascular repair mechanisms in patients with permanent hyperglycemia. Indeed these changes could involve methylation in the promoter of the genes KDR and VEGF involved in the adhesion and angiogenesis.

60) Vitellogenin, Choriogenin, Cathepsin-L and Matrix Metalloproteinase-2 expression in Anchovy (*Engraulis ringens*)liver tissue

Fernández, F., Bustamante, S[1].,Barrientos, P[2].,Riquelme, O[1].,Reyes, C[1].,Morin, V[1].,Castro, L[2].,[1]Bioquimica y Biologia molecular, Ciencias Biológicas, Universidad De Concepción.[2]Oceanografía, Ciencias Naturales y Oceanograficas, Universidad De Concepción. (Financial Support: Fondecyt 1100534.).

Fishes are an important food source for humans, however, little is known about their reproductive biology, effects on their offspring and their relationship with the environment. Among the most important commercial fishes in Chile we can found the Anchovy (*Engraulis ringens*), a small pelagic species inhabiting from Peru (6°S) to the Patagonia (47°S), a wide latitudinal range where environmental conditions vary markedly.To understand the relationship between the reproductive biology of females and their effects on their offspring in different environmental conditions, a study in liver tissue of adult anchovies from the area of Talcahuano was carried out to determine the expression levels of the genes Vitellogenin, Choriogenin, Cathepsin-L and matrix metalloproteinase-2 (MMP-2), important in embryonic development. Primers were designed from mRNA sequences from species such as *Engraulis japonicus*, *Danio rerio*, *Oryzias latipes*, among others. The results of PCR assays show that the genes of these four proteins are expressed in the liver of the anchovy.Simultaneously, remarkable enzymatic activity of the proteins Cathepsin-L and MMP-2 was detected in liver tissue by a zimogram assay. These results are the first step to determine the expression and function of important proteins in embryonic development, first in mature females and then in eggs.

61) The Non-Coding mitochondrial RNAs regulate cell proliferation/apoptosis balance players

Fitzpatrick, C[1,2]., Briones, M[1,2].,Vidaurre, S[1].,Oliveira-Cruz, L[1].,Burzio, L[1,2].,Burzio, V[1,2].,[1]Andes Biotechnologies S.A. Fundación Ciencia y Vida.[2]Facultad de Ciencias Biológicas Universidad Andrés Bello. (Sponsored by Grants: PhD Scholarship, Conicyt; Fondecyt 1110835 And 1140345, Conicyt, Chile And INOVA-Corfo 12IEAT-16317)

The family of non-coding mitochondrial RNAs (ncmtRNAs) which displays differential expression between cancer and normal cells has been studied in our laboratory as a tool to generate a selective cancer therapy. Knockdown of ASncmtRNAs in mouse and human cancer cell lines induces massive apoptotic death, without affecting viability of normal cells. In addition, knockdown of ASncmtRNAs potentiates apoptotic cell death by inhibiting Survivin expression, a member of the inhibitor of apoptosis (IAP) family, and by relocalization of Bcl-2 to the nucleus. Apoptosis is preceded by cell proliferation inhibition and cell cycle arrest, displaying differential downregulation of Cyclins B1 and D1 in tumor cells. Interestingly, the same treatment induces selective upregulation of cyclin A1 and CDK2 in normal cells. These features can be explained by a mechanism involving putative new mitochondrial micro RNAs (Mito-miRs) generated from the ASncmtRNA. The selective tumor cell death by this approach is brought about by a simultaneous “attack” on different fronts, showing promise for the application of this molecular target for an efficient and safe cancer therapeutic strategy.

62) Dissecting the function of the DEAD-box RNA helicase DDX3 on HIV-1 genomic RNA translation

Fröhlich, Á[2]., Rubilar, P[1].,Rojas, B[2].,Ohlmann, T[1].,Soto-Rifo, R[2].,[1]International Center for Infectiology Research Université de Lyon.[2]Instituto de Ciencias Biomedicas, Virología, Medicina, Universidad De Chile. (Sponsored by Fondecyt 11121339)

DDX3 is a host factor essential for HIV-1 replication and thus, a potential target for novel therapies aimed to overcome viral resistance. We have previously shown that DDX3 plays a critical role during translation initiation of the HIV-1 genomic RNA (gRNA). As such, DDX3 binds to the gRNA and destabilize the TAR RNA motif allowing the recognition of the m^7GTP cap structure and the engagement of the viral RNA in cap-dependent translation initiation. Interestingly, this process seems to occur in cytoplasmic granules where the gRNA accumulates with DDX3 and translation initiation factors eIF4GI and PABP. Although the function of DDX3 during HIV-1 translation requires its catalytic activity, it is unknown whether domains surrounding the catalytic core are involved. Here, we have conducted an analysis in order to determine the involvement of the N- and C-terminal domains of DDX3 in regulating HIV-1 gRNA translation. Our results suggest that the N-terminal domain of DDX3 is intrinsically disordered and contains three putative, non-canonical RNA-binding motifs involved in the assembly of the gRNA in cytoplasmic granules and translation. Interestingly, overexpression of the N-terminal domain of DDX3 resulted in the specific inhibition of gRNA translation. Interestingly, we observed that inhibition of Gag expression by the N-terminal domain of DDX3 was conserved in the closely related lentivirus HIV-2 suggesting a conserved role that could be exploited in the development of novel anti-HIV drugs aimed to counteract the generation of viral resistance.

63) Statistical potentials to evaluate the binding affinity for NAD(P) in proteins

Fuentealba, M[1]., Cabrera, R[1].,[1]Departamento de Biología, Facultad de Ciencias, Universidad De Chile. (Sponsored by FONDECYT 1121170.)

The nicotinamide adenine dinucleotides, NAD and NADP (NAD(P)), are cofactors involved in the production and consumption of reducing power for the generation of energy and biosynthesis in all organisms. The NAD(P) binding proteins are generally highly selective, being able to discriminate for the presence of the 2'-phosphate group in otherwise structurally equivalent cofactors. Since the level of affinity towards NAD or NADP depends on the specific interaction between atoms in the protein and the ligand, we aimed for a better understanding of the recognition determinants in NAD(P) binding proteins through the derivation of statistical potentials, generated from the whole repertory of known NAD(P) complexes. A protein database was built by selecting non-redundant and high resolution structures that bind NAD(P), showing a complete molecule in extended conformations. Statistical potentials were generated for 12 atom types representing the 20 proteinogenic aminoacids and 10 atom types for NAD(P). Thereafter, we used a set of 51 protein-cofactor complexes to correlate their known experimental binding energy with their score obtained after applying the statistical potential, at different bin sizes and cutoff distances. We obtained a maximum correlation coefficient of 0.76 for NAD and 0.74 for NADP. We also studied through statistical potentials, the effect of mutations affecting the specificity for NAD(P) in order to evaluate its application in predicting the preference for these cofactors in enzymes.

64) Evolution of transcriptional responses of the Muscle Ring Finger Protein (MURF) family in muscle in salmonids after two extra rounds of whole genome duplication (WGD)

Fuentes, E[1]., Valdes, J[1].,Molina, A[1].,Johnston, I[2].,Macqueen, D[3].,[1]Biotecnologia Molecular, Ciencias Biologicas. INCAR (Interdisciplinary Center for Aquaculture Research) , Universidad Andrés Bello.[2]School of Biology, Scottish Ocean Institute, University of St Andrews.[3]Institute of Biological and Environmental Sciences University of Aberdeen. (Sponsored by MASTS VF20, FONDAP INCAR 15110027 And FONDECYT 1130545)

Introduction: WGD was experienced two times more by salmonids than other vertebrates. To understand the role of paralogues genes in muscle growth we studied the MURF family, which are key regulators of protein degradation on atrophy.

Methods: Exhaustive bioinformatics screens of nuclear genomes and in-house transcriptome assemblies identified 9 unique salmonids *murfs* paralogues genes: 3 *murf1*, 4 *murf2*, 1 *murf3* and 1 novel *murf4*. The transcriptional responses of all *murfs* repertory were analyzed in atlantic salmon (*S. salar*) subjected to glucocorticoids (GC) treatment, and coho salmon (*O. kisutch*) and rainbow trout (*O. mikiss*) subjected to bacterial infection.

Results: All *murfs* paralogues were expressed in muscle in all salmonid species with exception of *murf2b2*. In atlantic salmon all *murf1* paralogues were upregulated with the GC treatments, but the rest components were not affected. In coho salmon and rainbow trout, all *murf1* paralogues were upregulated after bacterial infection. Only *murf2a1* was upregulated in rainbow trout; whereas all *murf2* paralogues were upregulated in coho salmon after treatment. *murf3* and *murf4* mRNA levels were not affected. **Discussion:** Paralogues genes have evolved separately showing differential transcriptional responses to different treatments. Particularly, *murf1* (*a1, b1, b2*) and *murf2* (*a1*) paralogues would have an important role in muscle atrophy under catabolic conditions.

65) Atomic Force Microscopy studies on the mechanical properties of endothelial glycocalyx-like layer

Fuentes-Cassorla, C[1]., Navarrete, C[1].,Mansilla, L[2].,Poblete, I[1].,Torres, S[2].,Figueroa, X[1].,Barrera, N[1].,[1]Physiology, Biological Sciences, Pontifica Universidad Católica de Chile.[2]Faculty of Engineering Universidad De Valparaíso. (Funded By Millennium Science Initiative P10-035F, Fondecyt 1120169 And Anillo ACT-1108 Grants.)

Endothelial cells (ECs) form a syncytium located in the blood vessels luminal side. This cellular type is regulated by many mechanical stimuli such as shear stress, cellular contact and changes in systemic blood pressure. ECs are extracellularly coated by the glycocalyx, a soft matter thick layer composed mainly by glycoproteins. All cell interactions and pharmacological effects have to pass through glycocalyx layer prior to contact the EC plasma membrane. Currently, studies on the EC glycocalyx have been focused on the layer composition, but its mechanical properties are poorly understood. Using a new Atomic Force Microscopy (AFM) approach on primary ECs culture we have measured the glycocalyx stiffness, damping, indentation and Young Modulus. According to our measurements the glycocalyx thickness is about 0,7 µm in the highest part of the cell and this value decreases at the cell periphery. Moreover, we have determined that the glycocalyx has a very low stiffness and high damping.

66) Upregulation of DISC1 and its association with ribosomal protein S6 in response to cellular stress

Fuentes-Villalobos, F[1]., Farkas, C[1].,Pincheira, R[1].,Castro, A[1].,[1]Laboratorio de Transducción de Señales y Cáncer, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad De Concepción. (Sponsored by FONDECYT 1120923)

DISC1, a protein involved in neuronal development has been extensively studied for its association with schizophrenia and other mental disorders. However, the underlying mechanisms of DISC1 function remain elusive. It has been implicated in several signaling pathways, including the regulation of the Akt-mTORC1 signaling, which promotes cell growth and survival mainly due to stimulation of mRNA translation. Consistently, DISC1 interacts with the p40 subunit of the eukaryotic translation initiation factor 3. DISC1 is recruited into stress granules (SGs), structures of translationally-stalled mRNAs and proteins which are assembled after different stress inputs. This observation has been interpreted as a role for DISC1 in the regulation of the translation of mRNAs that are not recruited to SGs. Here, we analyze a possible role of DISC1 in the regulation of protein synthesis in response to genotoxic and metabolic stress. We found that DISC1 expression is upregulated after these treatments in different cell lines. In addition, we have found that DISC1 interacts with ribosomal protein S6, another protein taking part in the preinitiation complex of protein translation. Our results support a role for DISC1 in the regulation of protein synthesis under stress conditions shared in neurodegenerative diseases and tumour microenvironment.

67) Insights into the oxidation mechanism of veratryl alcohol and its role as redox mediator in lignin peroxidase from Phanerochaete chrysosporium: a theoretical study

Fuenzalida, I[1]., Recabarren, R[1].,Alzate-Morales, J[1].,[1]Centro de Bioinformática y Simulación Molecular, Facultad de Ingeniería, Universidad De Talca. (Sponsored by J.A.M. And I.F. Thank Financial Support Through Project FONDECYT No. 1140618.)

Veratryl alcohol (VA) is a secondary metabolite of the fungus *P. chrysosporium* and is the main substrate of lignin peroxidase. VA acts as a redox mediator enhancing the oxidation of lignin model compounds and after being oxidized to a radical species by residue Trp171, it serves as an oxidant to degrade lignin and other substrates. However, it is not well understood if VA either remains attached to the protein surface close to Trp171, or acts as a diffusible oxidant through the lignin matrix. In this work the electron transfer mechanisms between Trp171 and VA, and the stabilization of the VA+ species at the protein surface, were studied by means of different computational techniques. Docking experiments were used to identify the most probable conformations of VA around Trp171, which were then used as starting point to perform MD simulations. Results showed that hydrogen bonds with Asp264 and π-π stacking interactions with Phe267 represent crucial interactions for VA binding. The importance of Phe267 was probed by in silico mutations corroborating earlier experimental findings. MD simulations revealed a highly occupied region of the enzyme, which we propose could correspond to an allosteric site for VA. Finally, snapshots from MD were used in order to perform QM calculations. Spin density distributions showed that a frontal position of VA, with respect to Trp171, is needed for the electron transfer to take place. We expect these results lead us to future experimental work at our lab, which will be focused on site-directed mutagenesis.

68) Deregulated expression of the drug transporters ABCB3, ABCC3, SLC3A2, SLC28A1 and SLC29A1 is associated with a drug-resistant phenotype of gallbladder cancer cells.

García, P[1]., Bizama, C[1].,Espinoza, J[1].,Leal, P[2,3].,Weber, H[2].,Alfaro, F[4].,Apud, MJ[1].,Riquelme, I[2].,Romero, D[4].,Roa, JC[5].,[1]Departamento de Anatomía Patológica, CITO, Facultad de Medicina, Pontificia Universidad Católica De Chile.[2]Departamento de Anatomía Patológica, CEGIN-BIOREN, Facultad de Medicina, Universidad de La Frontera.[3]McKusick-Nathans Institute of Genetic Medicine, School of Medicine, Johns Hopkins University.[4]Departamento de Anatomía Patológica, Facultad de Medicina, Pontificia Universidad Católica de Chile.[5]Departamento de Anatomía Patológica, CITO, FONDAP ACCDIS, Facultad de Medicina, Pontificia Universidad Católica De Chile. (Funded By FONDECYT 11130515 And 1130204.)

Gallbladder cancer (GBC) is a highly aggressive disease and intrinsically resistant to chemotherapy, but mechanisms involved in drug resistance have not been completely elucidated. The aim of this study was to evaluate the transcriptional expression of drug transporters belonging to the ABC (ATP-binding cassette) and SLC (solute carrier) families in GBC cell lines with a multidrug-resistant phenotype. Chemosensitivity (based on drug dose response curves) was determined by exposing the GBC cells to gemcitabine (GEM), cisplatin (CDDP) and 5-Fluorouracil (5-FU) for 72 h. Further, relative expression of 16 drug transporters was determined in sensitive and resistant cells, and potential candidates were evaluated in clinical samples. Differential chemosensitivity was found between cell lines, but TGBC-1TKB (lymph node metastases) and G-415 (derived from ascites) showed to be resistant to all chemotherapeutic drugs tested. OCUG-1 (established from peritoneal effusion) was highly sensitive to GEM and 5-FU, but resistant to CDDP. qRT-PCR analysis showed increased levels of ABCB3, ABCC3 and SLC3A2 in drug resistant cell lines, while SLC28A1 and SLC29A1 transcripts were down-regulated. In tissue samples, these genes were also differentially expressed in tumors versus non-neoplastic tissues. Our findings suggest a potential contribution of these drug transporters to the intrinsic chemoresistance of GBC.

69) Role of S6K1 in insulin-mediated changes on mitochondrial function in cultured cardiomyocytes

García, I[1]., López-Crisosto, C[1].,Parra, V[1,2].,Lavandero, S[1,2].,[1]Departamento de Bioquímica y Biología Molecular Universidad de Chile.[2]Department of Internal Medicine, Southwestern Medical Center University of Texas. (Sponsored by FONDAP 15130011 (SL), ACT 111 (SL), FONDECYT 1120212 (SL). IG And CLC Hold A PhD Fellowship From CONICYT)

The cardiac muscle requires a continuous and abundant supply of ATP. Due to this high energy demand, mitochondria play an essential role to achieve this requirement. The morphology and function of this organelle is dynamically changing. This implies that the mitochondria are melted and divided depending on the metabolic needs of the cell. Particularly, the fusion event is complex, because it must melt both outer and internal membranes, which are effected by Mfn1/Mfn2 and OPA1, respectively. Our recent work showed that insulin increased mitochondrial fusion by activating the signaling pathway Akt/mTORC1/OPA1 in cardiomyocytes. However, it remains unclear which is the effector downstream of mTORC1 that allows such changes. Because mTORC1 phosphorylates and activates S6K1, we test the hypothesis here that S6K1 mediates the increase in OPA1. To this end, cardiomyocytes were treated with insulin 10 nM, 3h. As previously shown this hormone increased OPA1 and mitochondrial membrane potential and promoted mitochondrial fusion in cardiomyocytes. However, all these effects were abolished when the cells were pretreated with a chemical inhibitor for S6K1 before exposure to insulin. The upstream effector of insulin signaling pathway Akt was not altered by the S6K1 inhibitor. These results suggest that S6K1 could play a key role in the effects of insulin on mitochondrial function and morphology in cardiomyocytes.

70) Protective role of insulin/Akt/p65NFkB signaling pathway in ischemic cardiomyocytes.

Garcia, P[1]., Diaz, A[1].,Humeres, C[1].,Gomez, M[1].,Gonzalez, V[1].,Lavandero, S[1,2].,[1]Advanced Center for Chronic Diseases (ACCDiS) and Center for Molecular Studies of the Cell (CEMC) Faculty of Chemical and Pharmaceutical Sciences & Faculty of Medicine, University of Chile.[2]Department of Internal Medicine UT Southwestern Medical Center, Dallas. (Supported By FONDECYT 1110346, FONDAP 15130011 And ACT1111 (to LG, SL) And CONICYT PhD Fellowship 21110381 (to AD).)

Insulin controls key cardiac functions such as energy metabolism, muscle contraction and cell survival. The transcription factor NFκB seems to be protective against ischemia, however, both its role on the insulin signaling pathway and its target genes are still poor understood. To test these, cultured rat cardiomyocytes were treated with or without insulin (10 nM) and then submitted to simulated ischemia for 8 hours. Cytoprotective effects of insulin were measured by Trypan blue exclusion, LDH release, flow cytometry and TUNEL. Protein levels were measured by Western blot. Our results showed that Insulin prevented cardiomyocyte death induced by ischemia. We found that insulin stimulates a higher and complete activation of Akt during ischemia. This effect was abolished by the treatment with Akt inhibitor (5 uM). We observed that insulin decreases IκBα levels and stimulated p65-NFkB translocation to nucleus. We concluded that insulin prevented and reduced cell death in ischemic cardiomyocyte by inhibiting apoptosis and necrosis. We also found that ischemia made cardiomyocytes more sensitive to insulin. These effects seem to be mediated by NFκB signaling pathway. These findings provide new evidence that insulin signaling pathway is sensibilized in ischemic cardiomyocytes.

71) LIMCH1 isoforms I and II: characterization of novel proteins with agmatinase activity. (LIMCH1 isoformas I y II: caracterización de dos nuevas proteínas con actividad agmatinasa).

García, D[2]., Torrealba, N[2].,Órdenes , P[1].,Benitez, J[2].,García, M. D. L. Á[1].,Carvajal, N[2].,Uribe, E[2].,[1]Departamento de Biología Celular, Facultad de Ciencias Biológicas, Universidad De Concepción.[2]Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad De Concepción.

Agmatine (decarboxylated arginine) has functions as neurotransmitter, modulation of the insulin release from pancreatic cells and renal sodium excretion and enhancement of the analgesic effect of morphine. Agmatine is degraded to putrescine and urea by agmatinase, which has been cloned from bacterial and animal tissues. In contrast with well characterized bacterial species, the knowledge of the mammalian enzyme is very scarce, because they express a very low, if any, activity *in vitro*. In our laboratory we have cloned and characterized a rat brain protein with agmatinase activity but not belonging to the ureohydrolase family. This agmatinase like protein (ALP) contains 523 aminoacid residues and database analyses indicate that it is part of the carboxyl extreme of a putative protein denominated LIMCH1, with two isoforms of 1085 (I) and 902 (II) aminoacid residues. Both proteins contain a LIM-domain in his carboxyl extreme (characteristic of ALP) and isoform I also presents a calpoin homology domain in its amino extreme. In this study, we have cloned and expressed the isoforms I and II of LIMCH1. Both proteins resulted to be active as agmatinase and were dependent on Mn^{2+} for catalytic activity. *Km* values were 5 mM and 1.8 mM for the isoforms I and II, respectively. K_m and k_{cat} values were essentially equal to those previously determined for ALP. These findings reinforce the importance of these proteins for regulation of the cellular concentrations of agmatine in mammals. Fondecyt 1120663.

72) Acute stress produce changes in specific microRNAs levels that targets genes coding for key proteins involved in neuroplasticity

García-Pérez, M[1]., Muñoz-Llanos, M[1].,Vidal , E[2].,Moyano, T[2].,Gutiérrez, R[2].,Pacheco, A[1].,Aguayo, F[1].,Fiedler, J[1].,[1]Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad De Chile.[2]Genética Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica De Chile. (Sponsored by FONDECYT 1120528)

Neuroplasticity refers to the ability of brain to change as a result of experiences allowing the reorganization of neural connections. It has been linked to memory and learning consolidation, and these processes involve variation in neural proteins. In this context, MicroRNAs (miRNAs) become relevant because these small non-coding RNAs regulate post-transcriptionally silencing or degrading mRNAs by matching a seed sequence in 3'UTR. Recently, we determined by microarray that depending on the duration of the stressor some miRNAs fluctuate in dorsal hippocampus. The aims of this study were to (i) verify by RT-qPCR whether those variations prevail in total hippocampus during and after acute restraint stress session (ii) evaluate the putative mRNAs target relevant for brain function by bioinformatics analyses. Adult male rats were stressed during 0.5 or 2.5 h and sacrificed immediately after restraint session, or 6 and 24 h post stress. We determined that miR-152 and miR-15b did not change in our model. Nonetheless, miR-92a showed a significant increase (2-4 folds) during the stress session (0.5-2.5 h) and a further increase (5 fold) was observed 6h post-stress. However a recovery to controls was detected 24 h post-stress. Interestingly, it has been demonstrated that miR-92a has a relevant role in status epilepticus and memory contextual fear memory. Thus, it is imperative to determine the role of miR-92a in stress response.

73) Construction of hybrid topology files for the unnatural amino acid homoarginine useful for free energy perturbation calculations

Gonzalez, F[1]., Poblete, H[2].,Caballero, J[2].,[1]Centro de Bioinformatica y Simulacion Molecular, Facultad de Ingenieria, Universidad De Talca.[2]Centro de Bioinformatica y Simulacion Molecular Universidad De Talca.

Molecular dynamics (MD) is a method useful for the study of the atomistic properties and functions of biological systems. MD methods allow the characterization of the atomistic biomolecule movement considering the Newton second law, and uses as input the information gathered in MD force fields (topology and parameters). Free energy perturbation (FEP) is a method derived from MD used, inter alia, to study the effect of amino acid (AA) mutations. A FEP study requires the information contained in MD force fields but some modifications are necessary in the topology information. In classic MD, the topology discloses the information of atomic charges and the presence of bonds, angles, and impropers, but FEP needs additional information related to conversion between one AA to the other, which is named hybrid topology. In general, the MD force fields include topological information for all the common biomolecules, including the natural AAs. However, this information is absent for unnatural AAs. In this work we elaborated the MD force field topology files (under the CHARMM force field) for the unnatural AA homoarginine, including classic and hybrid topologies, to perform FEP calculations. The reported topology files can be used to study the thermodynamic impact of mutations through free energy calculations with the software NAMD. We tested the quality of the novel topology files in the study of the mutation of arginine to homoarginine in solvent. In the future, we will use these files to study this mutation in peptides that act as protein kinase A inhibitors.

74) Determination of LIM-domain interaction proteins and analysis of possible Mn^{2+}coordinating residues in ALP.

González, D., Quiñones, M[1].,Benítez, J[1].,Cofré, J[1].,Carvajal, N[1].,Uribe, E[1].,[1]Bioquímica y Biología Molecular, Ciencias Biológicas, Universidad De Concepción.

Agmatinase catalyzes the hydrolysis of agmatine into putrescine and urea. Agmatine, is fundamental in neurotransmission, analgesia, polyamines biosynthesis and cellular proliferation. We have cloned a cDNA that encodes a protein with agmatinase activity, not belonging to the ureohydrolases superfamily of proteins, denominated agmatinase like protein (ALP). The sequence of ALP do not contain residues which are typical of the ureohydrolase family and serves for binding of the activating Mn^{2+}ions (His and Asp mainly). In addition, ALP exhibits a C-terminal LIM-domain that coordinate two Zn^{2+} ions. LIM-domain deletion of ALP generate an increase in its catalytic activity and we have gthrough this domain. Using the TAP-TAG strategy we have identified some proteins that interact with the LIM-domain and are potential regulators of the agmatinase activity of ALP. We have also analyzed the participation of His residues in the interaction of ALP with Mn^{2+}. Kinetic parameters for thewild-type, and histidine mutants (H65A, H127A, H206A, H394A and H435A) were essentially the same and in only one case (H206A) the *Kd* for Mn^{2+} was significantly increased. Finally, we have shown that wild type and mutants were further activated by incubation with $MnCl_2$ 2mM at 60ºC. We propose that. in spite of the absence of typical Mn^{2+} ligands, fully activated species of ALP contain a Mn^{2+} center, and that His residues are not relevant forMn^{2+}interactions in this enzyme. Fondecyt 1120663.

75) Analysis of the last common ancestor of bifuctional hydroxymethyl-pyrimidine/pyridoxal kinases enzymes.

González-Feliú, E[1]., Castro-Fernandez, V[1].,Guixé, V[1].,[1]Departamento de Biología, Facultad de Ciencias, Universidad De Chile. (Sponsored by Fondecyt 1110137).

The vitamin kinase family of the ribokinase superfamily has groups of enzymes that phosphorylate either hydroxymethyl pyirimidine (HMP) or pyridoxal (PL). Also, two bifunctional enzymes have been described with the ability of phosphorylate both HMP and PL, thereby forming a new subclass of enzymes called HMPK/PLK. These enzymes are present in Bacillus subtilis and Staphylococcus aureus. Previously, we described that this subclass derived from enzymes that are specific for HMP and that the last common ancestor between specific and bifunctional enzymes was promiscuous for PL. In this work we updated the phylogenetic tree of the family and constructed it by two methodologies, Maximum Likelihood and Bayesian inference. The overall topology of the trees differs between these two methods; however, the sub-topologies corresponding to the bifunctional enzymes are the same and in both cases present a highstatisticalsupport (98-100%). We inferred the ancestral sequence of the common ancestor of this subclass of enzymes (ancB) either by the Empirical Bayes methodology which is based on the tree obtained by the Maximum Likelihood method, and by Hierarchical Bayes, which use the populations of trees obtained by Bayesian inference. Sequences of ancB showed a difference of 10% over the two methodologies, but the active site residues, evidenced by homology models, are conserved. The idea that these enzymes have arisen from the HMPKs enzymes is confirmed and also pointed out that the most relevant mutation for bi-functionality is the change of GLN for MET.

76) Microsatellite identification for genetic variability analysis in Red cusk-eel (*Genypterus chilensis*)

González, P[1]., Estrada, J[2].,Gallardo, C[3].,Valdes, J[4].,Meneses, C[5].,Molina, A[1].,[1]Biotecnología Molecular, Ciencias Biológicas. INCAR (Centro interdisciplinario para la investigación acuícola), Universidad Andrés Bello.[2]Centro de Investigación Marina Quintay (CIMARQ) Universidad Andrés Bello.[3]Biotecnología y genómica acuática , INCAR (Centro interdisciplinario para la investigación acuícola), Universidad De Concepción.[4]Biotecnología Molecular, Ciencias Biologicas. INCAR (Centro interdisciplinario para la investigación acuícola), Universidad Andrés Bello.[5]Biotecnologia Vegetal Universidad Andrés Bello. (Sponsored by FONDAP INCAR 15110027)

Introduction: Low levels of genetic diversity can trigger problems such as mortalities or malformations. This is particularly important to establish the fish farming of new species. Microsatellites are motifs of nucleotides that repeat in tandem. They are codominant markers, highly polymorphic with mendelian inheritance, wildly used to determinate genetic variability of populations. In consequence, we search for highly polymorphic microsatellites in a population of red cusk-eel (*G. chilensis*).

Methods: Identification of polymorphic microsatellites was performed using the SSRlocator software. Fluorescent PCR products, obtained by conventional PCR, were used to analyze 110 fish from the CIMARQ Center. The fragments were analyzed in an ABI3037XL sequencer. Heterozygosity, PIC value and Hardy-Weinberg equilibrium were estimated using the GenePop software.

Results: The red cusk-eel transcriptome has 7% of genes that contains microsatellites. By comparing three different red conger transcriptomes, 30 polymorphic microsatellites were found. Within them, 12 were highly polymorphic.

Discussion: Identification of highly polymorphic microsatellites and the currently evaluation of genetic variability, are important steps in order to allow the establishment of a commercial farming of the red cusk-eel. Polymorphic microsatellites allow selecting broodstock with highly genetic variability in order to avoid undesired effects.

77) Transcriptional regulation of the zinc transporter coding gene *VvZIP3* during grapevine flower development.

Saavedra, G[1]., Roa, R[1].,Yañez, M[1].,Ruiz, S[1].,González, E[1].,[1]Instituto de Ciencias Biológicas Universidad De Talca. (Sponsored by Fondecyt 1120871).

Several grapevinecultivars shows tendency to develop parthenocarpic seedless grapes reproductive disorder originated in defective ovule fertilization due to a failure in pollen tube growth. Zinc deficiency, element required by "zinc finger" transcription factors involved in pollen development, has been invoked as one of the causes of this phenomenon. Zn uptake and its mobilization to aerial plant tissues involve several ZIP-type transporters. *VvZIP3,* a ZIP transporter encoding gene, is the only expressed during flower development. Regulatory elements recognized by MADS transcription factors associated to floral organogenesis control and responsive elements for ABA and GA, hormones implicated in grapevine reproductive development, have been detected in its promoter. To assess the involvement of MADS factors in the *VvZIP3* expression, deletions to remove the regulatory elements in the promoter region were generated, deleted fragments were fused to the *GUS* reporter gene and the constructs were used to transform *A. thaliana. GUS* expression was analyzed by qRT-PCR and histochemical staining. To evaluate the role of plant hormones on *VvZIP3* expression, applications of ABA, GA and NAA were performed on flowers at pre-bloom. Transcriptional activity of *VvZIP3* in response was determined by qRT-PCR. The obtained results indicate that *VvZIP3* expression is induced by both MADS transcription factors and the ABA-mediated signal transduction pathway.

78) Some extracts of native trees Chile induce tumor cell death by apoptosis in a gastric cancer cells lines.

González, C[1]., Marchant, M[1].,Greeley, A[1].,Vinet, R[2].,Tarnok, M[1].,Olivero, P[3].,Guzmán, L[1].,[1]Instituto de Quimica, Facultad de Ciencias, Pontificia Universidad Católica De Valparaíso.[2]Laboratorio Farmacología, Facultad de Farmacia, Universidad de Valparaíso.[3]Laboratorio de Biología Molecular , Facultad de Medicina, Universidad de Valparaíso. (Sponsored by 037.227/2014; 037.728-11 DI-PUCV.)

In recent years, a wide range of secondary metabolite plant physiological and pharmacological properties have been evaluated as antitumoral, anti-inflammatory and hypolipemiant agents. Phytochemicals, a form of plant-derived compounds include tannins, flavonoids, quinones, etc. In Chile a prominent source of bioactive compounds are present in *Quillaja saponaria*, an endemic tree of the central zone. Since, gastric cancer (GC) is a principal cause of cancer mortality in Chile, we explore if some extracts from *Quillaja saponaria* (QS) shown anticancer activity. The effect of extracts QS on the viability of SNU1 and KATO III of GC cell lines was evaluated by MTS assay. The apoptosis was analyzed by activation of caspase 3/7 and fragmentation of DNA assay by TUNEL. Additionally, in order to evaluate apoptosis the same extracts were tested with trypan blue and DIC microscopy using CHO cells as control.All the extracts evaluated exhibit antiproliferative activity. The most potent extract was E2, showing an 88% cell death and complete incorporation of trypan blue in 90% of cell cultures. Additionally, the extracts promoted apoptosis via caspase-3 and-7 in both CG cell lines. DIC microscopy technique found damage in the cell membrane in a dose that showed significant antiproliferative effect. This result was confirmed by measurement LDH activity.According to our results the Extract evaluated shown a significant antiproliferative effect, possibly through the activation of apoptosis.

79) Angiotensin-(1-9) is a safe anti-cardiac hypertrophy peptide

Ocaranza, M. P[1]., Oyarzun, A[2].,Chiong, M[2].,Lavandero, S[2].,[1]Enfermedades Cardiovasculares, Facultad Medicina, Pontificia Universidad Católica de Chile.[2]Advanced Center for Chronic Diseases (ACCDiS), Facultad Ciencias Quimicas y Farmaceuticas, Universidad de Chile. (Sponsored by FONDEF D11I1122 (MPO; SL; MC), FONDAP 15130011 (SL; MC))

Angiotensin-(1-9) [Ang-(1-9)] is a novel peptide in the non-canonical renin-angiotensin system. Recently we showed that this peptide prevents and reduces cardiac hypertrophy triggered by myocardial infarction or hypertension. This effect was direct on cardiomyocytes and not mediated by Ang-(1-7). In this study, we investigated whether Ang-(1-9) is safe new anti-cardiac hypertrophy peptide. To this end, we evaluated the effects of Ang-(1-9) on the cell viability and proliferation as well as in basal apoptosis in cultured cardiomyocytes. Our results showed that cell viability and cell cycle was not modified in cardiomyocytes treated with increasing concentrations of Ang-(1-9) [1 nM-1 mM] for 24-72 h. With regards to the effect of Ang-(1-9) on basal apoptosis, Ang–(1–9) did not change the number of basal apoptotic cells at different doses and times evaluated. In conclusion, Ang-(1-9) did not stimulate cell death by necrosis neither basal apoptosis in cultured cardiomyocytes. Ang-(1-9) is a safe new anti-hypertrophy cardiomyocyte agent for the treatment of the cardiac pathologies.

80) microRNAs can regulate the differential expression of Ezh1 and Ezh2 during hippocampal neuron maturation.

Guajardo, L[1,4]., Aguilar, R[1,4].,Gutierrez, R[2,3].,Van Zundert , B[1].,Montecino, M[1,4].,[1]Center for Biomedical Research, Departamento de Ciencias Biológicas, Facultad de Ciencias Biológicas y Facultad de Medicina, Universidad Andrés Bello.[2]Biochemistry and Molecular Genetics, Biological Sciences, Pontificia Universidad Católica De Chile.[3]FONDAP Center for Genome Regulation, Santiago, Chile Pontificia Universidad Católica De Chile.[4]FONDAP Center for Genome Regulation, Santiago, Chile Universidad Andrés Bello. (Sponsored by FONDECYT 1130706 FONDAP 15090007 FONDECYT 3140418).

Ezh1 and Ezh2 are the catalytic subunits of the Polycomb Repressive Complex 2 (PRC2), which mediates epigenetic control of target gene transcription in a wide number of cell types. Ezh1 and Ezh2 (mRNAs and proteins) are differentially expressed during maturation of hippocampal neurons; Ezh2 is mostly expressed in immature neurons, whereas Ezh1 is principally expressed in mature neurons. As there is evidence from other cell systems indicating that expression of Ezh1 and Ezh2 can be regulated by miRNAs, we assessed whether the differential expression of these PRC2 catalytic subunits during hippocampal maturation is regulated by specific miRNAs present in these neurons. We first analyzed the global transcriptome and microRNA expression profile of immature (5DIV) and mature (20DIV) rat hippocampal neurons using Exon and miRNA Arrays (Affymetrix). We find a differential expression pattern of miRNAs during this neuronal maturation process and, importantly, that some of them have the ability for targeting the 3'-UTRs of both Ezh1 and Ezh2 in immature and mature neurons. Additionally, luciferase gene reporter-based functional assays confirm the presence of miRNAs in these types of neurons that are capable of down regulating the expression of Ezh1 and Ezh2. Together, our results indicate that the expression of Ezh1 and Ezh2 can be regulated by specific microRNAs that are differentially expressed during hippocampal neuron maturation.

81) Characterization of the plant root hair elongation inhibitor RH26

Guajardo, Á[1]., Rodríguez-Furlán, C[1].,Norambuena, L[1].,[1]Centro Biología Molecular Vegetal, Facultad de Ciencias, Universidad De Chile. (Sponsored by FONDEF-IDeA CA12I10206).

Plant root hairs (RH) are tubular structures that develop postembryonically at the elongation zone of root and they are important to increase the surface for plant nutrient uptake. RH elongates from the specialized trichoblast cells by tip growth. RH elongation depends on multiple hormonal factors and polarized membrane trafficking. Perturbation of these processes originates feasible phenotypes. These characteristics make RH an excellent system for studying tip growth. By means of Chemical Genomics, a strategy that looks for chemical that perturb biological processes, we have found a set of chemicals that could be useful as biological tools. Among them we are characterizing the effect of the chemical RH26. This chemical inhibits RH elongation by a dose-response effect. RH26 does not induce any change in the primary root length suggesting that its effect is specifically for RH growth. Nevertheless RH26 also affects the gravitropic root orientation. RH26 phenotypes can be linked to alterations in the levels of the hormone auxin. Actually RH26 causes an alteration in the normal auxin accumulation pattern in roots compared with untreated plants. Moreover, we have found that RH26 affect the accumulation of auxin transport facilitator PIN2 at the plasma membrane. Overall, these results suggest that RH26 most likely affects auxin polar transport that regulates auxin level affecting specifically trichoblast developmental processes in *A. thaliana*.

82) Interleukin-8 promotes microvascular permeability through eNOS activation.

Guequén, A[1]., Zamorano, P[1].,Rebolledo, L[1].,Burboa, P[2].,Quezada, C [3].,Ehrenfeld, I[4].,Sarmiento, J[5].,Sánchez, F[1].,[1]Inmunología, Medicina, Universidad Austral De Chile.[2]Fisiología, Medicina, Pontificia Universidad Católica De Chile.[3]Bioquímica, Ciencias, Universidad Austral De Chile.[4]Histología y Patología, Medicina, Universidad Austral De Chile.[5]Fisiología, Medicina, Universidad Austral De Chile.

Pro-inflammatory mediators increase microvascular permeability by eNOS activation and nitric oxide (NO) production. Recently, it has been described that NO promotes S-nitrosation of proteins from the adherens junctions resulting in destabilizing of this complex leading to hyperpermeability. Interleukin-8 (IL-8), the main pro-inflammatory agent in humans, is secreted by glioblastome and breast tumor cells. In the case of glioblastome IL-8 promotes increase in endothelial permeability associated with VE-cadherin internalization. The aim of this study was to determine the role of eNOS activation in microvascular permeability induced by IL-8 present in conditioned medium (MC) from glioblastome cells (U87) and breast tumor cells (MCF-7).

As a model we use EA.hy926 immortalized endothelial cells treated with IL-8, U87-CM and MCF-7-MC. Endothelial permeability was measured through flux of dextran-FITC-70 in cellular monolayers. eNOS activation was evaluated through western-blot and S-nitrosation of VE-cadherin and p120 was measured by biotin-switch assay.

IL-8, U87-CM and MCF-7-MC increased vascular permeability. This increase was inhibited in the presence of MAB208 (blocking antibody of IL-8). IL-8 also induced eNOS phosphorylation. IL-8, U87-CM and MCF-7-CM induced Ve-Cadherin and p120 S-nitrosation. These results indicate that IL-8 present in U87-CM and MCF-7-CM increase microvascular permeability through eNOS activation and S-nitrosation of adherens junction proteins.

83) Caveolin-1 enhanced internalization of gold nanoparticles in B16F10 melanoma cells as an approach to permit cell tracking *in vivo*.

Guerrero, S[1]., Diaz, V[1].,Hassan, N[2].,Guzman, F[3].,Kogan, M[2].,Quest, A[1].,[1]Laboratorio de Comunicaciones Celulares, Centro de Estudios Moleculares de la Célula (CEMC), Centro de Estudios Avanzados en Enfermedades Crónicas (ACCDiS), Programa de Biología Celular y Molecular, Instituto de Ciencias Biomédicas (ICBM), , Facultad de Medicina, Universidad de Chile.[2]Laboratorio de Nano biotecnología, Centro de Estudios Avanzados en Enfermedades Crónicas (ACCDiS),, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.[3]Núcleo de biotecnologia Curauma (NBC), Facultad de Ciencias Básicas y Matemáticas , Pontificia Universidad Católica de Valparaíso. (Sponsored by FONDECYT 1130250 (AFGQ); FONDECYT 1130425 (MK), FONDECYT 1140926 (FG), ACT1111 (AFGQ), CONICYT-FONDAP 15130011 (AFGQ, MK), CONICYT Postdoctorado 3140463 (SG); CONICYT Postdoctorado 3140489 (NH), CONICYT Student Fellowship (VD).)

Introduction:

Our group has shown that Caveolin-1 (Cav-1) increases migration, invasion and metastasis of B16F10 melanoma cells. However, to date evidence indicating precisely which step is modulated by Cav-1 in metastasis *in vivo* is not available due to the inability to track these cells real time once injected into the animals.

Here we describe advances on obtaining the cells labeled with nanoparticles (NP) without modulating functional parameters, such as viability and cell migration *in vitro*, as the first essential step focused in the near future to label and track cells in our animal model with the objective of unraveling Cav-1 function in metastasis.

Materials and methods:

Gold nanoparticles (12nm) were conjugated to peptide sequences that facilitate cell penetration (CR7, Cys-$TAT_{(48-60)}$ and $R_{(7)}$CLPFFD). B16F10 cells transfected with pLacIOP-Cav-1 (B16F10/Cav-1) or pLacIOP (B16F10/mock) were treated with NP and evaluated for viability (MTS), migration (transwell assays) and internalization (confocal microscopy) using NP labeled with Alexa-647.

Results and discussion:

The presence of Cav-1 was found to enhance the labeling of cells, while parameters such as viability and migration were not modified. These observations indicate that these peptide-modified NP can be employed to efficiently label cells without modifying important biological parameters and that such cells may be employed for tracking cells *in vivo*.

84) Efficient large-scale detection of structural relationships in proteins

Gutiérrez, F[1,2,3]., Rodriguez, F[1].,Melo, F[1,2].,Devos, D[4,3].,[1]Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile.[2]Molecular Bioinformatics Laboratory Millennium Institute on Immunology and Immunotherapy.[3]Centre for Organismal Studies Heidelberg University.[4]Centro Andaluz de Biología del Desarrollo CABD Universidad Pablo de Olavide. (Sponsored by FONDECYT 1141172, ICM P09-016-F And Heidelberg University Frontier Grant: 28577 Project #: D.801000/12.074)

The total number of known three-dimensional protein structures is rapidly increasing. The need for fast structural search against the complete database is also more demanding. Recently, an fast method for finding rigid structural relationships between a query structure and the complete Protein Data Bank (PDB) has been released. However, accurate and comparably efficient flexible structural aligners to perform whole database searches are not yet available. Here we report on the development of a new method for the fast and flexible comparison of protein structures. The method relies on the calculation of 2D matrices containing a description of the 3D arrangement of secondary structure elements. The comparison involves the matching of an ensemble of substructures through a nested-two-steps dynamic programming. The unique features of this new approach are the integration and trade-off balancing of the following: speed, accuracy and flexible substructure matching. The search of one medium sized (250-aa) query structure against the complete PDB database takes about 8 min in an average performance desktop computer. The method is able to detect partial structure matching, rigid body shifts, conformational changes and tolerates substantial structural variation arising from insertions, deletions and sequence divergence. We validate the performance of the method for fold assignment in a large benchmark set of protein structures. We finally provide a series of examples to illustrate the usefulness of this method and its application in biological discovery.

85) Bioinformaticsevaluation: use of MM-GBSA and APBS reproducing the binding free energies of XTH enzyme with different fibers polymers

Carrasco, Cristian[1]., Valenzuela, C[1].,Moya-León, M[1].,Herrera, R[1].,[1]Instituto Ciencias Biológicas Universidad De Talca. (Sponsored by Fondecyt N° 1120635. CC Received A Universidad De Talca Scholarship.)

Many studies have showed that xyloglucan endotranglucosydase/hydrolase (XTH) enzyme act mainly in the assembly and disassembly of the plant cell wall, allowing development, growth and cell elongation. The radiata pine XTH1 protein (Pr-XTH1) interacts with many kinds of hemicellulose sustrates, but the preference substrate is still unknown. The prediction union type and energy stability of Pr-XTH1 enzyme against different substrate (XXXGXXXG, XXFGXXFG, XLFGXLFG and GGGGGGGG) was determined by using bioinformatics tools. Molecular Docking, Molecular Dinamics, MM-GBSA and Electrostatic Potential Calculations were used to predict the binding modes, free energies of interaction and electrostatic charge distributions. The results suggest that the enzyme presented more stability with hemicellulosic substrates and the best stability was for xyloglucan complex XXXGXXXG substrate with -63.12 +/- 0.52 Kcal/mol. During molecular dynamics trajectories, hemicellulose fibers showed a greater degree of positional stability in relation to the substrate cellulose type (2 to 4 Å of RMSD). ΔΔG interaction obtained with MM-GBSA showed that Van der Walls force was the most relevant interaction energy in all cases. Finally, the more electronegative charges are located in the middle of the enzyme where the catalytic site interacts with substrates. This bioinformatics approach allows us to predict the enzyme favoritism to different substrates.

86) Transcriptional control of glutaredoxin *GRXC9* expression by a salicylic acid-dependent and NPR1-independent pathway in *Arabidopsis*

Herrera-Vásquez, A[1].,Carvallo, L[1].,Salinas, P[1].,Loreto, H[1].,[1]Genética Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica De Chile. (Supported By FONDECYT (1141202) And Millennium Nucleus For Plant Functional Genomics (P10-062-F))

Salicylic acid (SA) is a key hormone that mediates genes transcriptional reprogramming in the context of the defense response to stress. *GRXC9*, coding for a CC-type glutaredoxin from *Arabidopsis*, is a SA-responsive gene induced early and transiently by an NPR1-independent pathway. Here, we address the mechanism involved in this SA-dependent pathway, using *GRXC9* as a model gene. We first established that *GRXC9* expression is induced by UVB exposure through this pathway, validating its activation in a physiological stress condition. *GRXC9* promoter analyses indicate that SA controls gene transcription through two *as-1*-like elements located in its proximal region. TGA2 and TGA3, but not TGA1, are constitutively bound to this promoter region. Accordingly, the transient recruitment of RNA polymerase II to the *GRXC9* promoter, as well as the transient accumulation of gene transcripts detected in SA-treated WT plants, was abolished in a *knock out* mutant for the TGA class II factors. We conclude that constitutive binding of TGA2 is essential for controlling *GRXC9* expression, while binding of TGA3 in a lesser extent contributes to this regulation. Finally, over-expression of *GRXC9* indicates that the GRXC9 protein negatively controls its own gene expression, forming part of the complex bound to the *as-1*-containing promoter region. These findings are integrated in a model that explains how SA controls transcription of *GRXC9* in the context of the defense response to stress.

87) Biochemical characterization of a novel mesophilic ADP-dependent bifunctional phosphofructokinase/glucokinase from *Methanococcus maripaludis*

Herrera-Morandé, A[1]., Castro-Fernandez, V[1].,Moraga-Bravo, F[1].,Guixé, V[1].,[1]Biología, Ciencias, Universidad De Chile.

In some archaea, the phosphorylation of glucose and fructose 6-phosphate (F6P) is carried out by enzymes that are specific for their substrate and use ADP as a phosphoryl donor. However, in the hyperthermophilic archaeon from *Methanocaldococcus jannaschii* a bifunctional enzyme able to phosphorylate glucose and F6P was described. To determine if bi-functionality is a common feature for other enzymes of the order *Methanococcales*, we expressed, purified and characterized the unique homologous protein of the mesophilic archaea *Methanococcus maripaludis (Mm*PFK/GK*)*. From kinetic analysis with different sugars, metals and nucleotides we concluded that *Mm*PFK/GK is able to phosphorylate both F6P and glucose using ADP and a divalent cation. Also, *Mm*PFK/GK shows a complex regulation by free Mg^{2+} and AMP with the latter appearing to be a key metabolite. To address the possible role of *Mm*PFK/GK in glucose formation we evaluated the reversibility of both reactions and found that glucokinase activity is reversible, while phosphofructokinase activity is not. Residues involved in glucose and F6P binding were determined by modeling the *Mm*PFK/GK enzyme and its interactions with both sugar substrates using protein–ligand docking. Comparison of the active site of the *Mm*PFK/GK enzyme with the structural models constructed for all the homology sequences of the order *Methanococcales* shows that all of the ADP dependent kinases would be able to phosphorylate glucose and F6P, which rules out the current annotation of these enzymes as specific phosphofructokinases. Fondecyt 1110137

88) Alteration in effector immune cells and in Treg cells correlate with an increased b16 tumor growth in P2x7 knockout mice.

Ibañez, J[1]., Montoya, M[1].,Mena, J[1].,Escrig, D[1].,Michelson, S[1].,Acuña-Castillo, C[1].,Dante, M[2].,[1]Centro Biotecnológico Acuícola, Facultad de Química y Biologia, Universidad De Santiago De Chile.[2]Inmunobioquímica, Facultad de Química y Farmacia, Universidad De Chile. (Sponsored by Fondecyt 1110734 (CA), Fondecyt 11110401 (MM))

The P2X7 receptor is a member of the family of nonselective cationic channels gated by ATP. This receptor can sense extracellular ATP released from damaged or death cells playing an important role as mediators of the inflammatory response. Among the cell populations expressing this channel stand Treg lymphocytes and NK cells. Treg lymphocytes are able to inhibit the peripheral immune response, being an important mechanism of cancer cell escape to immune surveillance. P2X7 depletion in Treg induce an increase in apoptosis resistance, accumulating these cells in spleen, lymph nodes and peripheral blood. On the other hand, NK cells are characterized by their ability to kill tumor cells. So, we wanted to study the relationship between tumor development and the distribution of both immune cell populations in C57BL/6B6 P2X7 knock out mice. First we analyze the percentage of these cell populations in P2X7 KO mice. Our results indicated that there is an accumulation of Treg in secondary lymphoid organs and peripheral blood. On the opposite, we observed a decrease of NK cells in spleen and peripheral blood. When these KO mice were challenged with B16 melanoma cells, mice showed an increased tumor growth rate. Moreover, tumors shown a decrease in NK cells infiltrations. These results suggest that P2X7 have an important role in regulating the tissue distribution of NK and Treg cells favoring immune defense against tumor cells.

89) Cytotoxic activity of Flavobacterium psychrophilum in rainbow trout *(Onchorhynchus mykiss)* myoblast

Iturriaga, M[1]., Fuentes, E[2].,Carcamo, J[3].,Reyes, A[4].,Avendaño-Herrera, R[5].,Molina, A[2].,Valdés, J[1].,[1]Laboratorio de Bioquímica Celular, Facultad de Ciencias Biológicas. Interdisciplinary Center for Aquaculture Research (INCAR), Universidad Andrés Bello.[2]Laboratorio de Ciotecnología Molecular, Facultad de Ciencias Biológicas. Interdisciplinary Center for Aquaculture Research (INCAR), Universidad Andrés Bello.[3]Instituto de Bioquímica y Microbiología, Facultad de Ciencias. Interdisciplinary Center for Aquaculture Research (INCAR), Universidad Austral De Chile.[4]Laboratorio de Biología del desarrollo, Facultad de Ciencias Biológicas. Interdisciplinary Center for Aquaculture Research (INCAR), Universidad Andrés Bello.[5]Laboratorio de Patología de Organismos Acuáticos y Biotecnología Acuícola, Facultad de Ciencias Biológicas. Interdisciplinary Center for Aquaculture Research (INCAR), Universidad Andrés Bello. (Sponsored by CONICYT/ FONDAP/15110027 And Núcleo DI-447-13/N)

Introduction: *Flavobacterium psychrophilum* is one of the most important bacterial pathogens affecting salmonid freshwater. Infection of salmonid fish is generally associated with septicaemia and necrotic myositis. However, the invasive features of F. *psychrophilum* to muscle tissue and the pathogenesis of host cell death have not been thoroughly investigated. **Material and Methods:** cultured rainbow trout myoblast were infected with 4 different strains of *F. psychrophilum* at an MOI of 100 at temperatures of 15 or 18ºC under iron-limited conditions. Cytotoxic analyses were performed 24, 48 and 72 h after infection using LIVE/DEAD® Viability/Cytotoxicity Kit as well as DNA laddering and caspase activity. **Results:** Apoptosis was observed in rainbow trout myoblast upon infection, characterized by the occurrence of DNA ladder and the activation of caspase. The maximum apoptotic effect was induced by JIP strain 72 h after infection under iron-limited conditions. **Discussion:** The present study revealed that *F. psychrophilum* interacts with rainbow trout skeletal muscle and intrinsic pathways are activated in this cellular host to mediate cell death.

90) International scientific cooperation and programs of the German Research Foundation DFG

Kausel, G[1]., [1]Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral De Chile.

Science and research in Germany are characterised by an excellent infrastructure, a wide variety of disciplines, well-equipped research facilities and competent staff. The German Research Foundation, DFG (Deutsche Forschungsgemeinschaft) is the central public funding organisation for academic research in Germany with a Budget of about 2.5 Billion Euros in 2013. Its a member organization (universities, non-university institutions eg Max-Planck, Helmholtz, academies of science), promoting science and humanities in all branches. Its specific role lies in funding basic research at universities in all fields of science. DFG pays special attention to promotion and education of Young scientists and researchers. In all programs internationational cooperation is encouraged. In addition, DFG advices parliament and public authorities on research questions and suppports links between university and industry. Specific programs support initiation of international cooperation via mututal visits and bilateral workshops leading to joint project proposals. In the period of 2009-2013 DFG funds for chilean research projects reached 9.7Mio Euros, including individual research grants, mercator profesor, initiation activities, earthquake programe, package projects, research centers and particular joint research Project funding in Conicyt-DFG program. Thus, DFG programs promote and strengthen cooperation between the best researchers and sustainably strengthen cooperation with Chile in education, research and development.

91) Structural requirements of the human sodium-dependent bile acid transporter (hASBT): role of 3- and 7OH moieties on binding and translocation of

Lagos, C. F[1]., Gonzalez, P[2].,Ward, W[3].,Polli, J[4].,[1]Department of Endocrinology , School of Medicine, Pontificia Universidad Católica De Chile.[2]Department of Pharmacy, Faculty of Chemistry, Pontificia Universidad Católica De Chile.[3]DMPK Research and Development Scynexis Inc..[4]Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland. (Supported By FONDECYT Nº 11090199 And DIPOG-Facultad De Química PUC Grants To PMG. Molecular Dynamics Simulations Were Performed At The National Laboratory For High Performance Computing (NLHPC ECM-02) Supercomputing Infrastructure: Powered@NLHPC.)

Bile acids (BAs) are the end products of cholesterol metabolism. One of the critical steps in their biosynthesis involves the isomerization of the 3β–hydroxyl (−OH) group on the cholestane ring. BAs are actively recaptured from the small intestine by the human Apical Sodium-dependent Bile Acid Transporter (hASBT) with high affinity and capacity. The aim of this study was to elucidate the role of the 3α -OH group on BAs binding and translocation by hASBT. Ten 3β–hydroxylated BAs (Iso-bile acids, iBAs) were synthesized, characterized, and subjected to hASBT inhibition and uptake studies. hASBT inhibition and uptake kinetics of iBAs were compared to that of native 3α-OH BAs. Kinetic data suggests that, in contrast to native BAs where hASBT binding is the rate-limiting step, iBAs transport was rate-limited by translocation and not binding. Remarkably, 7-dehydroxylated iBAs were not hASBT substrates, highlighting the critical role of 7-OH group on BA translocation by hASBT, especially for iBAs. Molecular dynamics simulations and conformational analysis of gly-iBAs and native BAs identified topological features for optimal binding such as: concave steroidal nucleus, 3-OH on-or below-steroidal plane, 7-OH below-plane, and 12-OH moiety toward-plane. Our results emphasize the relevance of the 3α-OH group on BAs for proper hASBT binding and transport and revealed the critical role of 7-OH group on BA translocation, particularly in the absence of a 3α-OH group.

92) The molecular dynamics of Shaker and BK channels permeation and selectivity in atomistic detail using a double bilayer system.

Latapiat, V[1]., Sepúlveda, R[1].,González, F[2,1].,[1]Center for Bioinformatics and Integrative Biology (CBIB), Facultad de Ciencias Biológicas, Universidad Andrés Bello.[2]Centro Interdisciplinario de Neurociencias de Valpraiso Universidad de Valparaiso. (Acknowledgement: This Work Was Supported By FONDECYT Nº 1131003 And CINV (Millenium Initiative, 09-022-F).)

The role of potassium channel family is allowing the passage of K+ ions across cell membrane in order to elicit different cellular processes: generation and propagation of signal, gene expression regulation and neurotransmitters release. The potassium channel family allows fluxes of 106–108 K^+ ions per second, due to the presence of a structure called selectivity filter (SF) composed by a highly conserved sequence TVGYGD. Currently, the non-equilibrium molecular dynamics simulations using an external electric field have become a popular tool to study the permeation process in these channels. Nonetheless, the experimental realistic approach would be simulating the ion concentration gradients (Nernst gradients) across to a membrane, which drives an ionic flux across to channel. We built molecular systems including two lipids bilayer separated by aqueous compartments of similar size for the two K+ voltage-gated Shaker and BK channels. This study shows a computational technique that controls the ionic concentration gradient and the potential difference across the membrane during atomistic simulations. Moreover, a particle interchange method, which exchanges ion/water pairs between buffer regions in both compartments, was applied to maintain a given concentration gradient and/or charge imbalance. Finally, this study provides new perspectives to the ionic K^+ channels and can give insights to future experimental assays in other ion channels.

93) Nordihydroguaiaretic acid hampers glucose transport by directly blocking the human GLUT1 transporter

León, D[1]., Ojeda, L[1].,Pérez, A[1].,Zambrano, Á[1].,Salas-Burgos, A[2].,Reyes, A[1].,Salas, M[1].,[1]Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral De Chile.[2]Departamento de Farmacología, Facultad de Ciencias Biológicas, Universidad De Concepción. (Sponsored by FONDECYT 1130386, FONDEF D11I1131, DID-UACh S-2013-22)

Cancer cells have a high dependence on glucose metabolism; therefore, the pharmacological inhibition of glucose uptake by natural products is a promising strategy to hamper the growth of neoplastic cells by generating an energy-deprived state. Nordihydroguaiaretic acid **(**NDGA) is a polyphenol extracted from the bush *L. tridentata*, which has antioxidant and antiproliferative properties, and that is structurally similar to resveratrol (RSV), a non-competitive blocker of glucose transport facilitated by GLUT1 (*Salas et al. Am. J. Physiol. 305*: C90, 2013). Here, we analyze the action of NDGA on glucose transport facilitated by the GLUT1 carrier in human erythrocytes and the human leukemic U937 and HL-60 cell lines. Cytochalasin B displacement assays provides persuasive evidence that NDGA interacts directly with GLUT1. NDGA behaves as a non-competitive blocker of glucose uptake under zero-trans entry assays in U937 and HL-60 cells (IC_{50} 53 and 85 µM, respectively), suggesting that NDGA does not interact with the transporter's external ligand binding site. Besides, NDGA also hampers glucose transport in human erythrocytes under infinite-cis exit conditions (IC_{50} 26 µM). Likewise, Sen-Widdas assays suggest that NDGA displaced glucose from the external site of GLUT1. The results suggest that NDGA interacts directly with GLUT1 and behaves as a noncompetitive inhibitor of glucose uptake in U937 and HL-60 leukemic cell lines. Finally, we compare our kinetic results with docking simulations of NDGA and RSV binding to the 3D structural model of the GLUT1 transporter.

94) DNA-methyltransferase DNMT3a and RAC1 gene expression are increased in hypertensive patients

Lizama-González, J[1]., Carvajal, C. A.[1].,Reyes, M[1].,Valdivia, C[1].,Campino, C[1].,Lagos, C. F.[1].,Vecchiola, A[1].,Allende, F[2].,Solari, S[2].,Baudrand, R[1].,Fardella, C. E[1].,[1]Endocrinología, Medicina, Pontificia Universidad Católica De Chile.[2]Departamento de Laboratorios Clínicos, Escuela de Medicina, Pontificia Universidad Católica De Chile. (Sponsored by FONDECYT 1130427, SOCHED 2012-04 (CAC), FONDEF-IDeA CA12i10150, IMII P09/016-F (ICM) And CORFO 13CTI-21526-P1

We reported that 15% of essential hypertensives might suffer mineralocorticoid hypertension. CpG methylation by DNA-methyltransferases (DNMTs) can affect specific genes controlling the mineralocorticoid pathway either in renal and vascular tissues, as HSD11B2, MR and RAC1. DNMT3a and DNMT3b are involved in the *novo* methylation. **Aim**: To evaluate the 11BHSD2 activity *in vivo* and associate with expression of genes involved in the mineralocorticoid and methylation pathway. **Subjects and Methods:** We recruited 302 subjects, 111 hypertensives (HT) and 191 normotensives (NT) (Age 5-67 y-old). We measured folate, B12, serum aldosterone, plasma renin activity (PRA), cortisol (F) and cortisone (E) by LC-MS/MS. We isolated DNA and RNA from PBMC and evaluated the expression mineralocorticoid-pathway associated genes (HSD11B2, MR, RAC1, 18S) and DNA-methyltransferases (DNMT3a, DNMT3b, DNMT1) by qRT-PCR. **Results:** HT patients have higher F/E ratio (5.31 vs 4.95; $p<0.05$) than NT patients. We observed 16% more CpG methylation in HT compared with NT. Expression analyses showed similar HSD11B2 and MR expression in HT compared to NT, and higher expression of DNMT3a (7.1±1.6 vs. 2.4±0.6 AU $p<0.05$) and RAC1 (9.8±1.9 AU vs. 3.4±1.2 AU, $p<0.05$). DNMT3b expression increase with age (R=0,36, $p<0.05$) and DNMT1 decrease with age (R=-0,43, $p<0.05$). **Conclusions:** Gene expression analyses showed increases of DNMT3a and RAC1, which suggest that the *novo* methylation in hypertensive patients is improved, which may affects some key genes involved in the mineralocorticoid arterial hypertension.

95) A structural model of *Gracilaria chilensis* Core-membrane linker Rep domain

Macaya-Zapata, L[1,2]., Martínez-Oyanedel, J[1].,Bunster, M[1].,[1]Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.[2]Carrera de Bioquímica, Facultad de Farmacia, Universidad de Concepción. (Sponsored by Fondecyt Nº 113.0256)

In *Gracilaria chilensis*, phycobilisomes (PBS) are formed by a central Core of Allophycocyanin(APC) and Rods of Phycocyanin(PC) and Phycoerythrin(PE) that radiate from the Core. This protein complex is organized to harvest and transfer energy to Photosystem II. The Core-Membrane linker (L_{CM}) is a chromophorylated protein associated to the core and it has been described as the terminal energy acceptor. Sequence analysis of L_{CM} shows the presence of PBP-like domain, interrupted by an insertion known as PB-loop, three repeat domains (REP) and connecting sequences (ARM). REP domains are possibly involved in the interactions with APC and the assembly of the PBS core. The purpose of this work was to build a molecular model for the REP1 domain, a fragment composed by 130 amino acid residues, using the comparative modelling software MODELLER v9.13. and Molecular Dynamics in GROMACS v4.5.4. The best model generated was evaluated for stereochemistry and energy using PROCHECK and ProSA respectively. The model presents 62% of helical structure. The availability of a structural model will allow interaction studies with the PBS Core and understand the role of these domains in the assembly of this complex.

96) Structural and functional study of VvGST3, a grapevine gene encoding a putative glutathione S-transferase involved in flavonoid transport

Madrid-Espinoza, J[1]., Arenas-Salinas, M[1].,Ruiz-Lara, S[2].,[1]Escuela de Ingeniería en Bioinformática Universidad de Talca.[2]Instituto de Ciencias Biológicas Universidad de Talca. (Sponsored by FONDEF G07I1003)

Glutathione S-Transferases (GSTs) constitute a superfamily of proteins in prokaryotes and eukaryotes, involved in primary and secondary metabolism, detoxification of xenobiotics and defense against pathogens. They are divided into ten groups according to their percent identity, two of which, called tau and phi, are involved in the transport of flavonoids from the ER to the vacuoles for their storage. Among these, the Arabidopsis TT19 protein is involved in the transport of anthocyanins and proanthocyanidins (PAs). However, several of its counterparts described to date, as AN9 in petunia, BZ2 of maize, VvGST4 and VvGST1 in grapevine, are involved only in the anthocyanins transport. Herein, the protein VvGST3 was structurally analyzed and its gene functionally characterized. Two flavonoids binding sites were tested, one related to anthocyanins (Site A) and other to PAs (Site P), according the model to the TT19 protein. The role of the amino acid W203 also was evaluated through of W203L mutation. The results suggest that VvGST3 could transport anthocyanins and PAs. Functionally, the levels of transcripts of the *VvGST3* gene were determined in different vegetative tissues of grapevine and developmental stages of fruit grape. In addition, the capability of *VvGST3* to complement the mutant phenotype *tt19- 1* from *A. thaliana* was evaluated. The obtained results suggest that VvGST3 transport PAs but not anthocyanins during berry development in grapevine.

97) Homology Modeling of a Glutamate-gated chloride channel of *Caligus rogercresseyi:* An atomic-level perspective of interactions with Avermectins emamectin and ivermectin.

Maraboli, V[1].,Cornejo , I[2].,Andrini, O[3,4].,Niemeyer, M[2].,Teulon, J[3,4].,Sepulveda, F[2].,Cid, P[2].,Gonzalez-Nilo, F[1,5].,[1]Center for Bioinformatics and Integrative Biology, Facultad de Ciencias Biológicas, Universidad Andrés Bello.[2]Centro de Estudios Científicos (CECs) Valdivia, Chile..[3]UPMC Université Paris 06 UMR_S 1138, Team 3, F-75006, Paris, France.[4]INSERM UMR_S 872, Paris, France.[5]Centro Interdisciplinario de Neurociencia de Valparaíso Universidad de Valparaiso. (Sponsored by V.M. And F.D.G-N. Thank FONDECYT 1131003 And CINV (Millenium Initiative, 09-022-F). CECs Is Funded By Conicyt PFB)

Sea lice are marine ectoparasite copepods of the Caligidae family (order Siphonostomatoida) that attach to host marine fish and feed on their epidermal tissue and blood. Parasitic sea lice are a major sanitary threat to marine salmonid aquaculture with *Caligus rogercresseyi* as the principal sea louse species infesting farmed salmon and trout in Chile. Control of *Caligus* has been obtained with macrocyclic lactones (MLs) ivermectin and emamectin that target glutamate-gated chloride channels (GluCl) and act as irreversible non-competitive agonists causing neuronal inhibition, paralysis and death of the parasite. We have now cloned a full-length CrGluCla receptor from *Caligus rogercresseyi* that we show is irreversibly activated by ivermectin and emamectin. We have built a molecular homology model of CrGluClausing the crystal structure of a related GluCl channel of *Caenorabditis elegans* (PDBID 3RWH). Molecular Dynamics (MD) simulations give clues about the mode of action of ivermectin and emamectin in CrGluCla, allowing the identification of amino acids involved in the drug interaction and also the associated changes at the transmembrane portion leading to changes in pore diameter. The mode of interaction of MLs CrGluCla differs from that previously described in the crystallographic description supporting only a partially conserved mechanism of action.

98) Expression of a GH93 arabinanase from *Penicillium purpurogenum* in the methylotrophic yeast *Pichia pastoris* and its characterization

Mardones, W[1]., Callegari, E[2].,Eyzaguirre, J[1].,[1]Ciencias Biologicas Universidad Andrés Bello.[2]Proteomics Facility Universidad de Dakota del Sur. (Sponsored by FONDECYT 1130180; UNAB DI-31-12/I And DI-61-12/R; MECESUP UAB0802.)

Lignocellulose is part of the plant cell wall and consist of cellulose, xylan, pectin, and lignin. The enzymes involved in the lignocellulose degradation are important in different industrial processes. The filamentous fungus *P. purpurogenum* grows on different lignocellulosic carbon sources and secrete diverse types of enzymes for their breakdown. We utilized mass spectrometry (2D nanoLC MS/MS) to identify lignocellulolytic enzymes from supernatant of the fungus grown on sugar beet pulp. We identified 42 different putative enzymes, among them two putative arabinanases. The goal of this study is the biochemical characterization of one, ARAP2. For this purpose we heterologous expressed the *arap2* cDNA in the methylotrophic yeast *P. pastoris.* The cDNA was constructed from the *arap2* gene using "overlap-extension PCR". The cDNA was cloned in the expression plasmid pPICZB and *P. pastoris* was used as host. The recombinant ARAP2 was purified using a Ni affinity resin. The molecular weight (estimated using SDS-PAGE) spans 60-85 kDa, probably due to heterogeneous glycosylation. ARAP2 degrades debranched arabinan and has a broad range of pH optimum, between 4 and 6. The optimum temperature is near to 40º C. This is the first *P. purpurogenum* arabinanase characterized. The characterization of arabinanases allows for a better understanding of the lignocellulose degradation process.

99) Design of new nano-carriers based on bioinformatics analysis of protein-DNA interactions.

Marquez-Mmiranda, V[1]., Camarada, M.[2].,Araya, I.[2].,Almonacid, D.[1].,Gonzalez-Nilo, F.[1].,[1]Centro de Bioinformática y Biología Integrativa, Facultad de Ciencias Biológicas, Universidad Andrés Bello.[2]. Fraumhofer Chile Research (Sponsored by V.M.M Thanks To CONICYT Doctoral Fellowship. This Work Was Supported By Fraunhofer Chile Research, Innova-Chile CORFO (FCR-CSB 09CEII-6991) And Anillo Científico ACT1107.)

Dendrimers have gained prominence as efficient non-viral delivery carriers of drugs and nucleic acids, due to their unique features as well-defined size and shape, monodispersity and variable end-groups. Several efforts have been devoted to design a nanoparticle which can associate strongly enough to nucleic acids so that it remains intact during binding and entry into the cell. However, one of the issues that must be improved is how to modulate dendrimer - DNA interaction in order to promote the unpacking of the complex inside the cells, allowing the release of the cargo. Thus, we decided to employ the knowledge about how protein and nucleic acids interact in Nature with the goal of identifying the functional groups involved in these interactions. To gain insight into this matter, a bioinformatics strategy has been developed. By analyzing the Protein Data Bank, we have detected patterns in the interaction between proteins and nucleic acids. Using this platform, we have implemented a molecular design of new nano-carriers, called Synthetic Protein Based on Dendrimers (SPBD), which consists on a dendrimer-based nanoparticle with its surface conjugated with one or more amino acidic groups, which can act as a customizable gene carrier. By adjusting the type of amino acids, flexibility of the terminal groups and charge distribution, we can modulate the nucleic-acid binding properties of synthetic proteins. Thus, we describe Molecular Dynamics studies to characterize SPBD-DNA complexes, design new prototypes of SPBD, and improve their affinity for nucleic acids.

100) Efficient and automated large-scale detection of structural relationships in proteins with a flexible aligner

Gutiérrez, F[2]., Rodríguez-Valenzuela, F[2].,Devos, D[1].,Melo, F[2].,[1]Centro Andaluz de Biología del Desarrollo CABD Universidad Pablo de Olavide, Sevilla, España.[2]Genética Molecular y Microbiologia, Ciencias Biológicas, Pontificia Universidad Católica De Chile.

The total number of known 3D protein structures is rapidly increasing. The need for fast structural search against the complete database is also more demanding. Recently, an ultra-fast method for finding rigid structural relationships between a query structure and the complete PDB has been released. However, accurate and comparably efficient flexible structural aligners to perform whole database searches are not yet available. Here we report on the development of a new method for the fast and flexible comparison of protein structures. The method relies on the calculation of 2D matrices containing a description of the three-dimensional arrangement of secondary structure elements. The comparison involves the matching of an ensemble of substructures through a nested-two-steps dynamic programming algorithm. The unique features of this new approach are the integration and trade-off balancing of the following: 1) speed, 2) accuracy and 2) flexible substructure matching. The method is able to detect partial structure matching, rigid body shifts, conformational changes and tolerates substantial structural variation arising from insertions, deletions and sequence divergence, as well as structural convergence. We validate the performance of the method for fold assignment in a large benchmark set of protein structures. We finally provide a series of examples to illustrate the usefulness of this method and its application in biological discovery.

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101) Dinitrochlorobenzene-based immunotherapy against melanoma induces an increase in tumor-infiltrating T lymphocytes and Th 17 response

Mena, J[1]., Escrig, D[1].,Perez, D[1].,Mateluna, C[1].,Cardozo, Y[1].,Escobar, A[2].,Acuña-Castillo, C[1].,[1]Centro de Biotecnología Acuícola , Facultad de Química y Biología, Universidad De Santiago De Chile.[2]Facultad de Odontología Universidad de Chile. (Sponsored by FONDECYT 1110734)

Delayed type hipersensitivity (DTH) response, has been related to a positive prognosis in patients treated with immunotherapies against malignant melanoma. In account of this, we evaluated whether the DTH induction, with topical application of Dinitrochlorobenzene (DNCB), improved the antitumoral immune response, in melanoma B16 model. Mice were challenged with B16-Ovalbumin (OVA) alive cells and 3 days later, animals were sensitized with 2% DNCB in a different place of tumor challenge. Then animals were treated weekly on tumor challenged site with topical 0,1% DNCB. We analyzed the tumor growth, T lymphocyte populations in spleen and tumors, and specific T cell proliferation assay *in vitro* with OVA antigen challenge. Tumor detection was similar in both groups, near to day 15. However, treatment with DNCB delay the maximal tumor growth compared with excipient-treated group. Besides, we detected OVA specific $CD4^+$ cells proliferation *in vitro*, only in the DNCB treated group. Moreover, $CD4^+$, $CD8^+$ T regulatory (Treg) and T helper (h) 1 cells in spleen, did not show any significant differences between experimental groups, but treatment with DNCB induces an increase in Th17 cells level in spleen and $CD4^+$, $CD8^+$ lymphocytes infiltrating in tumor. In conclusion topical DNCB treatment induce an increase in Th17 response and specific $CD4^+$ antigen recognition, and enhance the infiltration of $CD4^+$ and $CD8^+$ cells. The protection against melanoma induced by DNCB could be used as an adjuvant therapy to generate immunity against melanoma.

102) *De novo* assembly and characterization of fine flounder (*Paralichthys adspersus*) transcriptome in different nutritional status using next generation sequencing

Mendez, K[1]., Zuloaga, R[1].,Valenzuela, C[1].,Fuentes, E[1].,Valdes, J[1].,Orellana, A[2].,Meneses, C[2].,Vizoso, P[3].,Molina, A[1].,[1]Biotecnología Molecular, Ciencias Biológicas. INCAR (Centro Interdisciplinario Para la Investigación Acuícola), Universidad Andrés Bello.[2]Laboratorio de Biotecnología Vegetal Universidad Andrés Bello.[3]Centro de Bioinformática Universidad Andrés Bello. (Sponsored by FONDECYT 1130545 And FONDAP INCAR 15110027)

Introduction: Fine flounder is an endemic species with economic importance for Chile. Nevertheless, almost no information is available about its biology. To increase aquaculture production, is necessary to understand the reprogramming of gene expression triggered by farming conditions, such as different nutritional status. Consequently, high-throughput muscle transcriptome sequencing from different nutritional states was performed.

Methods:Three paired-end libraries were generated from RNA samples obtained from muscle of fine flounder, which were subjected to 3 weeks of starvation then to 1 week of refeeding, also was defined a control prior to the start of the starvation, using MySeq of Illumina. RNA-seq analysis was performed using de novo transcriptome assembly with Trinity software.

Results: We obtained a total of 22 million reads, which were assembled into 93,317 contigs (N50=1,751bp). Were found 85,675 isoforms of transcripts and subsequently annotated to use as reference to perform a digital expression analysis. The transcript abundance from each library was calculated using RSEM software.

Discussion: We have produced a comprehensive first reference transcriptome of fine flounder. Our results provide a resource for future gene expression analysis, functional studies on production traits and improve our understanding of the biology of this species.

103) ATP and PMB-induced depletion of TREGS mediated by P2X7R. P2X7R knockout mice analysis

Lopez, X[1].,**Michelson, S[1]**., Mena , J[1].,Escrig, D[1].,Barrientos , C[1].,Faundez, A[1].,Sáez, J[2].,Imarai, M[1].,Acuña-Castillo, C[1].,[1]Centro de Biotecnología Acuícola, Facultad de Química y Biología, Universidad de Santiago de Chile.[2]Departamento de Fisiología Pontificia Universidad Católica de Chile.

Previously, we reported that regulatory T cells (Tregs) from C57BL/6 splenocyte preparation are depleted by the antibiotic polymyxin B (PMB) and ATP. A pharmacological approach associated this depletion to activation of pannexin-1 hemichannels or $P2X_7$ receptor ($P2X_7R$) activation. Now, our aim was to confirm the role of both membrane channels in ATP and PMB-induced depletion of Tregs.

Splenocytes from wild-type (WT), $P2X_7R$ KO and pannexin-1 KO C57BL/6 mice were challenged with different PMB and ATP concentrations. Twenty four hours later, splenocyte populations were studied by flow cytometry. In WT animals both ATP and PMB depleted Tregs in a concentration-dependent manner. Tregs from $P2X_7R$ KO mice were insensitive to ATP and PMB. On the other hand, Tregs from pannexin-1 KO animals were depleted only at high ATP concentrations, but were apparently insensitive to PMB. Dye uptake experiments in pannexin-1transfected HeLa cells show that PMB can't activate pannexin-1 hemichannels; however, in a CD4+-enriched splenocyte population, dye uptake was detected due to an activation of these hemichannels, suggesting the involvement of other actor - possibly P2X7R - in this effect. Together, our results suggest that $P2X_7R$ and pannexin-1 hemichannels are involved in ATP and PMB-induced depletion of Tregs. To corroborate these results, our perspective is to determine the effects of pannexin-1 hemichannels and $P2X_7$ inhibitors on Tregs-depletion in WT animals.

104) In vitro cytotoxic activity of the crude venom of the spider *Grammostola sp.* (Nortina) on mammalian cells and human carcinoma.

Mieres, D[1]., Araya, J[1].,Orrego, P[2].,Cornejo, M[3].,Ramirez, M[3].,[1]Laboratorio de Parasitología Molecular, Departamento de Tecnología Médica, Ciencias de la Salud, Universidad De Antofagasta.[2]Unidad de Biología Celular y Molecular, Departamento Biomédico, Ciencias de la Salud, Universidad De Antofagasta.[3]Laboratorio de Fisiología Celular y Molecular, Departamento Biomédico, Ciencias de la Salud, Universidad De Antofagasta. (Acknowledgements: Tutor Professor Dr. Jorge E. Araya Rojas. Faculty Of Health Sciences, University Of Antofagasta Chile And FONDEF IDeA CA12I10298 CONICYT.)

Objective:To characterize the components of the crude venom of the spider *Grammostola sp.* (Nortina) and evaluate their effect on cellular cytotoxicity in mammalian cells and human carcinoma. Methods: The poison is obtained by electrostimulation, filtered, quantified and stored at -80 ° C. Poison characterization was performed by SDS-PAGEs stained with Coomassie and silver nitrate, using Hemolysis assay protocol Hessinger and Lenhoff and protease activity assay using a gelatin zymogram and evaluated supplemented with different pHs. The effect of the venom on cytotoxicity in cell models was determined by measurement of LDH. **Results**:SDS-PAGEs the molecular mass of proteins and peptides of the poison (135-4 kDa) was observed. The hemolytic activity of the venom was zero in five concentrations compared to the positive control (Triton X-100). In the zymogram protease activity at pH 7.4 visualized, this has a molecular mass of 39 kDa. In the cultivation of mammalian cells (mouse peritoneal macrophages and MDCK cells) treated with poison, there was less cellular cytotoxicity compared to human carcinoma cells (A2780 and T-84) treated with crude venom, in both cell models observed a dose-dependent when comparing treated versus poison control (untreated cells) cell cytotoxicity.**Conclusion**: The crude venom of the spider *Grammostola sp*. (Nortina) is cytotoxic in mammalian cells and human dose-dependent carcinoma, presenting as an innovative anticancer agent in experiments in vitro effect.

Posters Session II

105) Determination of the three dimensional structure and characterization of threading mechanism of TraY, a protein with a probable knotted topology.

Molina, A[1]., San Martin, A[1].,Fuentealba, M[2].,Cabrera, R[2].,Baez, M[1].,[1]Departamento de Bioquímica y Biología Molecular, Laboratorio de Bioquímica, Facultad de Ciencias Químicas y Farmacéuticas , Universidad De Chile.[2]Departamento de Biología, Laboratorio de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad De Chile. (Sponsored by Fondecyt 11110534; Anillo ACT1107)

The RHH family of transcription factors is composed by homodimeric proteins formed by the association of two identical RHH motifs. However, the transcription factor TraY of *E. coli* is a monomer with two RHH motifs codified in a single polypeptide chain. Notably, homology models of TraY indicate that RHH domains fusion generates a knotted polypeptide chain whose structure and threading mechanism has not been experimentally determined. In order to confirm the presence of a knot we attempted to obtain the X-ray structure and performed unfolding experiments under equilibrium conditions using several mutants designed to prevent the threading of the polypeptide chain. Stability curves obtained with Guanidinium chloride, followed by intrinsic fluorescence and circular dichroism, showed similar conformational stability for TraY and mutants lacking 6 or 12 residues from the C-terminus. However, deleting 32 residues from the C-terminus created an unfolded protein under native conditions. To determine which terminus threads the polypeptide chain, TraY was fused to a hyperstable protein. The C-terminus fusion avoided the folding of TraY while the N-terminus fusion did not affect its structure. Moreover, TraY recovered its structure when the C-terminus fusion was cleaved. Considering these results, it is proposed that TraY present a knotted structure and that the threading of the polypeptide chain is guided by the C-terminus.

106) Binding modes of SB-206553 in different neuronal receptors (5-HT$_{2B/2C}$ and α7): an approach to rational design of poly-pharmacological drugs.

Möller-Acuña, P[1,3,4]., Reyes-Parada, M[2].,Contreras, J[4].,Alzate-Morales, J[3,4].,Rojas, C[4].,Iturriaga-Vásquez, P[5].,[1]Department of Biological Sciences, Faculty of Chemistry and Biology, Universidad De Santiago De Chile.[2]School of Medicine, Faculty of Medical Sciences, Universidad De Santiago De Chile.[3]Center for Bioinformatics and Molecular Simulation, Faculty of Engineering, Universidad De Talca.[4]School of Engineering in Bioinformatics, Faculty of Engineering, Universidad De Talca.[5]Department of Chemistry, Faculty of Science, Universidad De Chile. (Sponsored by FONDECYT Nº 1130185 MR-P, N° 1100542 PI-V., Doctoral Fellowship Awarded By CONICYT)

Psychiatric disorders incidence has grown steadily, but the complexity of the neural circuits underlying them has limited their physiopathological understanding.

However it is known that monoaminergic systems and neuronal nicotinic acetylcholine receptors play an important role in many of these disorders, which are the result of a complex network of molecular events, it is essential to develop drugs that act in a polyselective way. On the other hand, evidence from systems biology indicates that promiscuous drugs, are clinically better in terms of efficacy, than those that act in a more selective fashion.

In this work, we determined the putative binding modes of SB-206553, we employed the crystal of the 5-HT$_{2B}$ and the homology models of the 5-HT$_{2C}$ and α7-nAChR. Then, using docking and molecular dynamics methodologies, we established the most probable binding modes of the drug and analyzed the main molecular interactions involved, the new cavities were structurally compared looking for similarities.

Our results show a high structural similarity between binding sites the 5-HT$_{2Cand}$ α7, showing conserved residues. In addition, docking analysis and molecular dynamics show that the bond between the ligand with the cavities are mediated by hydrogen bonds, electrostatic interactions which determinate their selectivity.

We expect that such an analysis will serve to define the aspects underlying the affinity showed by SB-206553, and will also aid the rational design of novel compounds.

107) PDGF-BB induces mitochondrial degradation and autophagy in VSMCs.

Mondaca-Ruff, D[1]., Cartes-Saavedra, B[1].,Norambuena-Soto, I[1].,Vidal-Peña, G[1].,Morales, PE[1].,García-Miguel, M[1].,Pino-Espinoza, G[2].,Pedrozo, Z[2].,Lavandero, S[1].,Chiong, M[1].,[1]ACCDiS, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad De Chile.[2]ACCDiS, Facultad de Medicina, Universidad De Chile. (Sponsored by Fondecyt 1140329, FONDAP 15130011, Anillo ACT1111, D.M-R. Holds A Conicyt Fellowship)

Vascular smooth muscle cells (VSMCs) are an essential component of vessels involved in vascular tone regulation. During hypertension, atherosclerosis and diabetes, VSMCs change from a contractile to a proliferative phenotype. This change is associated to a metabolic shift from oxidative (mitochondrial) to glycolytic metabolism. Here we evaluate the effect of platelet-derived growth factor BB (PDGFBB) on mitochondrial degradation by autophagy as responsible for phenotype switching in VSMCs. Smooth muscle A7r5 cells from rat aorta were treated with PDGF-BB (10 nM) for 0-48 h. Autophagy was evaluated by LC3I/LC3II ratio and p62 in the presence and absence of chloroquine. Mitochondrial fragmentation was assessed by confocal microscopy and Mitotracker orange staining. PDGF-BB induced autophagy at 24 to 48 h. Chloroquine treatment suggests that this induction was due to *de novo* activation of autophagy. PDGF-BB induced mitochondrial fission at 3 to 6 h, as detected by an increase in the number of mitochondria with a simultaneous decrease in their volume. Our data suggest that PDGF-BB induces mitochondrial fragmentation followed by autophagy, probably to reduce the number of mitochondria in these cells. This process could play a key role in VSMC phenotype switching induced by PDGF-BB.

108) Diterpene phytohormone biosynthesis by two *Phaseolus vulgaris Rhizobium* symbionts

Montanares, M[1]., Díaz, W[1].,Méndez, C[1].,Baginsky, C[2].,Rojas, M[1].,[1]Química, Ciencias, Universidad De Chile.[2]Producción Agrícola, Ciencias Agronómicas, Universidad De Chile.

Gibberellins are a family of diterpene metabolites present as phytohormones in higher plants or as secondary metabolites in some fungal and bacterial systems. They derive from the precursor geranylgeranyl diphosphate which cyclizes to generate *ent*-kaurene, the first committed intermediate of GA biosynthesis. In contrast to plant and fungal systems few information is available about the enzymes and reactions of GA biosynthesis in bacterial systems. GA oxidase activities have been detected at significant levels only in bacteroids of *Bradyrhizobium japonicum*, a *Glycine max*. symbiont. This rhizobacteria contains an operon of GA biosynthesis genes that is expressed under the microaerobioc conditions found in root nodules of soybean plants. Products formed by the GA oxidases are non-hydroxylated GAs in contrast to plant and fungal oxidases that synthesize mainly 3β, 13-hydroxylated GA products.

In this work the enzyme activities of GA biosynthesis were investigated in bacteroids of two R*hizobium* species that are symbionts of *Phaseolus vulgaris*: *Rhizobium phaseoli* and *Rhizobium etli.* Bacteroids were obtained from symbiotic root nodules of plants inoculated with each of these species and grown under controlled conditions. ^{14}C-Labelled GA precursors were added to a bacteroid suspension and the metabolization products obtained after incubation were isolated and identified. High GA oxidase activities were found for both *Rhizobium* species but *R. etli* isolates showed a lower substrate utilization efficiency.

109) Degradation of neutral lipids contained in adiposomes is independent of autophagy in Sertoli cells

Montes De Oca, M[1]., Mancilla, H[1].,Covarrubias, A[1].,Cereceda, K[1].,Vander Stelt, K[1].,Angulo, M[1].,Slebe, J[1].,Concha, I[1].,[1]Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral De Chile. (Sponsored by FONDECYT 1110508 (IC), 1141033 (JCS))

In Sertoli cells ATP production mainly depends on lipid β-oxidation, these lipids can be stored in intracellular adiposomes or lipid droplets. Cytosolic lipases have a key role in neutral lipid catabolism contained in lipid droplets. It has been described that autophagy has a partial role in this process in various cell types, but it is unknown if this mechanism is present in Sertoli cells. In this study ATP content and both size and number of lipid droplets was evaluated in Sertoli cells under nutrient starvation treated with 3-methyladenine and bafilomycin A-1, chemical agents able to block early and late steps of autophagy, respectively. ATP content was determined by luminescence while the size and number of adiposomes was established by digital analysis of Oil Red O stained Sertoli cells images. It was observed that both 3-methyladenine and bafilomycin A-1 did not decrease the ATP content and did not affect both size and number of lipid droplets per cell. On the other hand the cholesteryl esterase activity inhibitor diethylumbellyferyl phosphate was able to increase the lipid droplet size (1,5µm^2 increase) and in addition with 3-methyladenine it was able to decrease the ATP content by 65%. This suggests that ATP production during nutrient starvation in Sertoli cells is independent of autophagy mediated degradation of neutral lipid stored in adiposomes.

110) Measurement of phagocytic activity and superoxide anion production in healthy and infected fish, with Infectious Pancreatic Necrosis virus (IPNv)

Morales, P[1]., Cardenas, F[1].,Yáñez, A[1,2].,Figueroa, J[1,2].,Haussmann, D[1,2].,[1]Instituto de Bioquímica y Microbiologia, Ciencias, Universidad Austral De Chile.[2]Interdisciplinary center for Aquaculture Research , (INCAR), Centro FONDAP.

Infectious Pancreatic Necrosis is a viral disease caused by the IPN virus, which infects salmonids, mainly Atlantic salmon causing high mortality. In fish head kidney occurs erythropoiesis, granulopoiesis and lymphopoiesis, and have a reticuloendothelial macrophages system. Spleen macrophages are involved actively generating material which can play an important role in immune memory.

In fish many type of cells are involved in cellular innate defense, variety of leukocytes, phagocytes including nonspecific cytotoxic cells. In this context, the phagocytic activity was evaluated in primary cultures of kidney macrophages, and superoxide anion production in head kidney cells from healthy and naturally IPNv infected fish.

Preliminary results indicate that phagocytic capacity increased considerably in IPN positive compared to healthy fish, which correlates with the increased production of superoxide anion in diseased fish (IPNv positive). Additionally, we use ELISA to evaluate the IgM antibody titre anti-IPNv. Results show a significant increase in the antibody titre in IPN positive asymptomatic fish, but decreases dramatically in fish with clinical symptoms.

These results could be classified into an innate immune response (early/late), which could be used to detect early immune response and disease progression, or if the fish are in the period in which they can receive treatment with satisfactory results.

FONDECYT 3130348, FONDAP 15110027 and Marine Harvest Company.

111) Effects of the infection with *Piscirickettsia salmonis* onto immunr response of atlantic salmon macrophages

Morales-Reyes, J[1,2]., Gonzalez-Bown, M[2,1].,Bastias, P[2].,Soto-Herrera, V[2].,Acuña, C[3].,Sandino, A[2,4].,Reyes-Cerpa, S[2,1].,[1]Laboratorio de Patogenos de peces Ictio Biotechnologies.[2]Laboratorio de Virologia, Quimica y Biologia, Universidad De Santiago De Chile.[3]Laboratorio de Genetica Humana, Quimica y Biologia, Universidad De Santiago De Chile.[4]Laboratorio de Virologia ActivaQ SA. (Sponsored by Proyecto CORFO Consorcio 13CTI-21527)

Piscirickettsia salmonis is facultative bacterium that infected and resides inside of salmonids macrophages. Mechanisms used by *P*. *salmonis* to survive and replicate within host cell are not fully understand. In this work, we analyze the transcript expression of markers of immune response when the bacterium infects macrophages in order to determine whether modulation of the host expression of pro and anti-inflammatory cytokines is associated with the bacterial survival.

Macrophages were isolated from kidney of *Salmo salar* by Percoll gradient and were characterized by microscopy and detection of specific molecular markers by PCR. Macrophages were challenged with *P. salmonis* and LPS from 24 to 96 hours. The transcript expression of TGF-β, IL-1β, IL-10, IL-12, IL-18, TNF-α, MHC-II, MHC-I and CD86 were evaluated by qRT-PCR and both condition compared.

Cytopathic effects were observed at 72 hours post-infection and intracellular bacterium was confirmed by TEM. Expression of early pro-inflamatory cytokines was similar in LPS- and *P. salmonis* stimulated macrophages. However, MHC-class II and CD86 transcription is slightly lower in macrophages infected by *P. salmonis*. In conclusion, *P. salmonis* infection not diminishes the expression of early inflammatory response, but decreases expression of markers of antigenic presentation, suggesting inhibition of macrophages function.

112) Analysis of the effects of overexpression of *SchSDD1* from *Solanum chilense* in stomatal frequency and tolerance to water deficit in *Arabidopsis thaliana.*

Morales-Navarro, S[1]., Verdugo, I[1].,Pérez-Díaz, R[1].,Ruiz-Lara, S[1].,[1]Instituto de Ciencias Biologicas Universidad de Talca.
(Sponsored by FONDEF D08I1118)

Drought is one of the most important factors affecting plant growth, development, survival and crop productivity. Physiological responses to drought include stomatal closure, decreased photosynthetic activity, altered cell wall elasticity, and even generation of toxic metabolites causing plant death. Plants also respond and adapt to water deficit at both the cellular and molecular levels. Control of this sequence of events using genetic engineering tools could result in improved plant stress tolerance. In order to restrict water loss, plants reducing the aperture of the stomatal pore or decreasing the number of stomata that form on the epidermis. Among the genes involved in the stomatal development, evidence exist that *SDD1* gene product of *Arabidopsis thaliana* acts in controling stomatal initial formation. Previous researches in our lab that plants of wild tomato (*Solanum chilense*) showed a reduction in stomatal numbers under salinity stress in new leaves. Therefore, it is tempting to postulate that the tomato homologous gene of *AtSDD1* would be involved in this survival strategy. Given this background, in this research the effects of overexpression of SchSDD1, a *Solanum chilense* putative ortholog gene of *AtSDD1*, on stomatal density and its ability to confer tolerance to water deficit in *Arabidopsis thaliana* plants were evaluated. The physiological parameters such as leaf RWC and electrolyte leakeage were measures in transgenic plants.

113) Exploring *the Arabidopsis thaliana* transcriptome state-space and their constraints.

Moyano, T[1]., Gutiérrez, R[1].,[1]Genética Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica De Chile. (This Work Is Supported By Grants From The International Early Career Scientist Programme From The Howard Hughes Medical Institute, FONDAP Center For Genome Regulation (15090007), Millennium Nucleus Center For Plant Functional Genomics (P10-062-F), FONDECY)

The transcriptome of an organism at a given developmental time and/or experimental condition is defined as a state. Cellular states evolve over development time or in response to environmental cues, allowing organisms to adapt over their life cycle. These adaptive changes are known to be partly mediated by changes in gene expression. In this work, we investigate the multidimensional space *S* of states that can be observed in an organism. We know that *S* has limits and that under normal conditions is not represented by a continuous function. We postulate that the different states *sj* represent homeostatic attractors operating under normal environmental conditions and that changes in a given state, for example in response to a perturbation, follow trajectories of low energy or high probability in *S*. We are determining the state spaces of *Arabidopsis thaliana* and the possible paths that connect these states when comparing different experimental conditions. Determining these states is a key element to understanding and predicting the rules that govern global changes in gene expression patterns in plants.

114) Molecular study of novel coumarin derivatives as selective acetylcholinesterase inhibitors

Muñoz, C[1]., Adasme, F[1].,Alzate, J[1].,Caballero, J[1].,Duarte, A[2].,Gutierrez, M[2].,Fonseca, A[3].,Joao-Matos, M[3].,[1]Centro de Bioinformatica y Simulacion Molecular, Facultad de Ingeneria, Universidad De Talca.[2]Laboratorio Síntesis Orgánica, Instituto de Química de Recursos Naturales, Universidad de Talca.[3]Departamento de Química Orgánica, Facultad de Farmacia, Universidad de Santiago de Compostela . (Sponsored by J.C, C.M And J.C Acknowledges The Financial Support From The Project FONDECYT No. 1130141 And No. 1140618.)

A wide range of coumarins derivatives and their biological properties are well known due to their synthetic accessibility, along with their abundant presence in plants and other natural products. These heterocyclic compounds have been previously related with broad biological activities like anticancer, antiviral, anti-inflammatory, antimicrobial, enzymatic inhibitory and antioxidant agents[1,2]. In particular, several coumarin-like compounds has been reported as acetylcholinesterase (AChE) inhibitors, enzyme that has been associated to the treatment of Alzheimer's disease. In light of this importance, we took some recently-synthesized in-house compounds (coumarin-amide and coumarin-quinoline) and evaluated them as potential inhibitors of AChE. We performed molecular docking studies to better understand the structure-activity relationships between the most active and inactive compounds against this enzyme. We further estimated the binding free energies by means of molecular dynamics simulations and MMGBSA calculations for certain molecules of particular interest. References 1.Borges, F. et al. Simple coumarins and analogues in medicinal chemistry: occurrence, synthesis and biological activity. Curr. Med. Chem. 2005, 12, 887-916. 2.Viña, D. et al. 3-substituted coumarins as dual inhibitors of AChE and MAO for the treatment of Alzheimer's disease. MedChemComm 2011, 3, 213-218.

115) Identification of new lncRNAs during osteoblast differentiation.

Nardocci, Gino[1,2]., Acevedo, E[1].,Nuñez, G[3].,Meneses, C[3].,Montecino, M[1,2].,[1]Center for Biomedical Research, Faculty of Biological Sciences and Faculty of Medicine, Universidad Andrés Bello.[2]Center for Genome Regulation FONDAP.[3]Center of Plant Biotechnology, Faculty of Biological Sciences , Universidad Andrés Bello. (Sponsored by FONDAP-15090007; FONDECYT-1130706; FONDECYT-3140414.)

Long noncoding RNAs (lncRNAs) are defined as untranslated RNA molecules, longer than 200 nt, 5`capped and polyadenylated, poorly conserved and that are linked to the regulation of chromatin structure, mRNA translation and control of gene transcription. Nevertheless, the mechanisms by which lncRNAs function to control gene expression are not yet understood. Recent advances in the methodologies utilized to analyze in depth the complexities of transcriptomes have unveiled the existence of new lncRNAs with relevant role in controlling gene expression during cell lineage commitment. In this study, mouse pre-osteoblastic cells were grown to confluence and then induced to differentiate into osteoblasts. At three sequential differentiation stages, total RNA was extracted using mirVana isolation kit and libraries were constructed using the Illumina TruSeq RNA sample preparation kit. The libraries were pooled and paired-end fragments were generated on an Illumina MiSeq sequencer. The resulting sequencing segments were aligned against the mouse genome with using Tophat2 and transcript abundances were determined by Cufflinks. New lncRNA candidates that displayed a differential expression pattern during the osteoblast differentiation process were identified and classified according to their expression profiles. This database will now allow genetic screen analyses seeking for regulatory lncRNAs that are capable of modulating mesenchymal cell lineage commitment decisions.

116) Herp regulates calcium signals in skeletal muscle cells

Navarro, M[1]., Díaz, F[1].,Troncoso, R[1].,Vásquez-Trincado, C[1].,Espinoza, S[1].,Jaimovich, E[2].,Lavandero, S[3,1,2].,[1]Advanced Center for Chronic Diseases (ACCDiS), Faculty of Chemical and Pharmaceutical Sciences & Faculty of Medicine, University of Chile.[2]Center for Molecular Studies of the Cell (CEMC), Faculty of Medicine, University of Chile.[3]Department of Internal Medicine (Cardiology Division) University of Texas Southwestern Medical Center. (Sponsored by FONDAP 15130011 (SL), ANILLO ACT111 (SL, EJ), CONICYT PhD Fellowship (MN))

Calcium ion plays a pivotal role in skeletal muscle physiology. Besides its role in muscle contraction, 1,4,5- inositol trisphosphate receptor (IP_3R)- dependent calcium transfer from endoplasmic reticulum (ER) to mitochondria regulates mitochondrial function. Herp (homocysteine- inducible ER protein), an ER membrane resident protein inducible under ER stress conditions, participates in the ER-associated protein degradation (ERAD) and protects the cell from ER stress-induced mitochondrial calcium overload and death in neuronal models. Besides its relatively high expression in skeletal muscle, the role of Herp in calcium homeostasis has not been evaluated. In this work we evaluate the role of Herp in cytoplasmatic and mitochondrial calcium response in rat L6 myotubes. Our results show that Herp knockdown did not affect cytoplasmatic calcium levels in basal conditions but increased histamine-induced cytoplasmatic calcium response. This correlated with an increase in IP_3R protein level. Interestingly, Herp knockdown decreased histamine-induced mitochondrial calcium response and decreased oxygen consumption in L6 myotubes. We concluded that Herp is required for an adequate calcium handling in skeletal muscle cells.

117) *In silico* studies of the permeation pathway of TRPV1 ion channels

Navas, C[2,3]., Sepulveda, R[3].,Baez-Nieto, D[3].,Brauchi, S[1].,Gonzalez-Nilo, F[2,3].,[1]Departamento de Fisiología, Facultad de Medicina, Universidad Austral De Chile.[2]Centro Interdisciplinario de Neurociencias de Valparaíso (CINV) Universidad de Valparaíso.[3]Center for Bioinformatics and Integrative Biology (CBIB), Ciencias Biológicas, Universidad Andrés Bello. (Sponsored by RVS Thanks To CONICYT Doctoral Fellowship. This Work Was Supported By FONDECYT 1131003(FGN), 1110906(SB), And CINV (Millenium Initiative, 09-022-F).)

TRPV1 ion channels are polymodal receptors classified into the superfamily of cationic channels TRPs (Transient Receptor Potential). The TRPV1 activation is mediated by a wide range of stimuli; including temperature (>42°C),capsaicin, extracellular acidification, lipids such as PIP_2 (phosphatidylinositol 4,5-bisphosphate) and changes in membrane voltage. The channel arranges as functional homotetramer, in which each subunit is formed by six transmembrane segments. Within the transmembrane region we can find two main structural features, is a ligand binding domain (VSD) formed by TM1 - TM4 segments, and apore domain constituted by TM5 - TM6 segments. The pore region contains an structure called P-helix, providing structural support to the selectivity filter.In order to study the pore/selectivity filter structure, we performed molecular dynamics simulations and analyzed the effect of several single and double mutations in TRPV1's P-helix. Our MD simulations on wt TRPV1 revealed one site of cation coordination. The consequences of mutations on P-helix (E636A, D646A, K639A, E636A/D646A, D646A/K639A) revealed, among other things, differences in the ion distribution into the selectivity filter and changes in the size of the pore of TRPV1.

118) GLP-1 prevents PDGF-BB induced dedifferentiation in VSMCs

Norambuena-Soto, I[1]., Cartes-Saavedra, B[1].,Morales, PE[1].,Mondaca-Ruff, D[1].,Nuñez-Soto, C[1].,García-Miguel, M[1].,-Mellado, R[2].,Lavandero, S[1,3].,Chiong, M[1].,[1]ACCDiS, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad De Chile.[2]Departamento de Farmacia, Facultad de Química, Pontificia Universidad Católica De Chile.[3]ACCDiS, Facultad de Medicina, Universidad De Chile. (Sponsored by Fondecyt 1140329, FONDAP 15130011, Anillo ACT1111. D.M-R. Holds A Conicyt Fellowship.)

Vascular smooth muscle cells (VSMCs) respond to changes in the local environment by adjusting their phenotype from contractile to synthetic, a phenomenon known as phenotypic modulation or switching. Platelet-derived growth factor-BB (PDGF-BB) is a potent inductor of contractile to synthetic phenotype switching and plays a key role in a number of major human diseases, including arteriosclerosis. Whether GLP-1, an incretin used in diabetes mellitus treatment, regulates PGDF-BB-dependent phenotypic switching in VSMC remains unexplored. Smooth muscle A7r5 cells from rat aorta were treated with GLP-1 (100 nM), PDGF-BB (10 nM) or GLP-1 + PDGF-BB. Phenotypic switching was evaluated by proliferation, migration and α-smooth muscle actin, SM22, smooth muscle myosin heavy chain and collagen I content. Our results showed that GLP-1 completely prevented PGDF-BB-dependent decrease in α-smooth muscle actin, SM22 and myosin heavy chain protein levels, as well as PGDF-BB-dependent increase of collagen I level. GLP-1 also blocked PDGF-BB-induced A7r5 migration, as determined by wound healing assay, and proliferation, as determined using MTT assay and cell cycle determination. Our data suggest that GLP-1 completely prevents the phenotypic switching induced by PDGF-BB.

119) High levels of aldosterone enhances SW872 preadipocyte differentiation

Gonzalez-Gomez, L. M[1]., Vecchiola, A[1].,Cifuentes, M[2].,Lagos, C[1].,Carvajal, C[1].,Allende, F[3].,Solari, S[3].,Campino, C[1].,Kalergis, A[4].,Fardella, C[1].,[1]Department of Endocrinology, School of Medicine, Pontificia Universidad Católica De Chile.[2]Institute of Nutrition and Food Technology (INTA) Universidad De Chile.[3]Department of Clinical Laboratories, School of Medicine, Pontificia Universidad Católica De Chile.[4]Millennium Institute Immunology and Immunotherapy, Faculty of Biological Sciences, Pontificia Universidad Católica De Chile. (Supported By SOCHED 2013-6, IMII P09/016-F, FONDEF CA12i10150, CORFO 13CTI-21526-P1 & FONDECYT 1130427 Grants.)

Overactivation of the mineralocorticoid receptor (MR) signaling by aldosterone has been associated with metabolic syndrome and adipocyte dysfunction. Our aim was to study *in vitro* if aldosterone determines changes in gene expression during adipogenesis. **Methods** SW872 preadipocytes were grown and differentiated using adipogenic cocktail (AC). Cultures undergoing differentiation were treated either with 1 or 10 nM aldosterone. Purified RNA from SW872 cell cultures was used for qRT-PCR and target gene expression during adipogenesis quantified by $2^{-\Delta\Delta Ct}$ method. **Results** Morphological changes were observed during AC treatment on cultures from 3 days on. CEBPβ expression increases 3 times over control after differentiation induction in control cultures (NT) as well as 1 nM aldosterone treated cultures. At day 6th after AC stimulation, cells treated with 10 nM aldosterone increased 13.3 times the CEBPβ expression over control. Treatment with either 1 or 10 nM aldosterone delayed 11β-HSD1 expression compared to control. Finally, PPARγ expression increased 2.4 times over basal expression 48h after adipogenesis in NT cultures. **Conclusions** *In vitro* studies on SW872 adipogenesis indicate that aldosterone enhances differentiation, as shown by higher lipid accumulation and differentiation markers expression, suggesting a differential key role for MR as a regulator of adipogenesis.

120) Biochemical and biopharmaceutical characterization of Angiontesin-(1-9)

Gonzalez, P[1].,Garcia, M[1].,Chiong, M[2].,Lavandero, S[2,3].,Jalil, J[4].,**Ocaranza, M[4].**, [1]Pharmacy Department, Faculty of Chemistry, Pontifical Catholic University of Chile. .[2]Advanced Center for Chronic Diseases (ACCDIS), Faculty Chemical & Pharmaceutical Sciences, University of Chile.[3]Department Internal Medicine, Cardiology Division, UT Southwestern Medical Center.[4]Cardiovascular Diseases Division, Faculty of Medicine, Pontifical Catholic University of Chile. (Sponsored by FONDEF D11I1122 (MPO, JJ, SL, MC), FONDAP 15130011 (SL, MC).)

Recent data from our laboratory have shown that angiotensin (Ang)-[1-9] protects from adverse cardiovascular remodeling in hypertension and myocardial infarction. However, little is known about biochemical and biopharmaceutical characteristics of Ang-(1-9) that are relevant for designing a drug product with potential pharmaceutical application. Our results showed that the isoelectric point for Ang-(1-9) was 7.6, with melting temperature in the range 160-164^{o}C, and decomposing around 210-209^{o}C. ^{1}H-, ^{13}C-, and two-dimensional NMR studies suggest a highly flexible, solvent-accessible structure. Ang-(1-9) was stable for up to 72 h in buffer and for no more than 3 h in the presence of MDCK cells (pH 5.5-7.4). Aqueous solubility of Ang-(1-9) at 37^{o}C was greater than 1 mg/mL (pH 1.2-7.4). Ang-(1-9) apparent permeability (Papp) ranged from 0.94 to 1.2 x 10^{-7} cm/s (pH 5.5-7.4), lower than that of pyranine (low permeability marker) which ranged from 3.1 to 4.2 x 10^{-7} cm/s. Finally, Ang-(1-9) Papp in PAMPA varied from 0.075 to 0.17 x 10^{-7} cm/s (pH 5.5-7.4). In conclusion, Ang-(1-9) was stable in aqueous buffer but unstable in presence of epithelial cells. Additionally, this peptide showed high water solubility and low permeability through both cell monolayers and artificial membranes. Results here presented suggest the needs for chemical and/or technological modification to improve the pharmaceutical profile of Ang-(1-9).

121) ISA virus regulates the generation of reactive oxygen species and p47phox expression in a p38 MAPK-dependent manner in *Salmo salar*

Olavarria, V[1]., Salas, B[1].,Villalba, M[1].,Sandoval, R[1].,Oliva, H[2].,Valdebenito, S[2].,Yañez, A[1,3].,[1]Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral De Chile.[2]Lonquén10.387, Maipú, Santiago Chile Veterquímica.[3]Víctor Lamas 1290, PO Box 160-C, Concepción, Chile Interdisciplinary Center for Aquaculture Research (INCAR). (Sponsored by FONDECYT N° 3120027, INNOVA-CORFO 12IDL2-16212)

Several viruses, including Orthomyxovirus, utilize cellular reactive oxygen species (ROS) for viral genomic replication and survival within host cells. However, the role of ROS in early events of viral entry and signal induction has not been elucidated. Here, we show that ISA virus (ISAV) induces ROS production very early during infection of CHSE-214 and SHK-1Ycells, and that production is sustained over the observed 24 h post-infection. The mitogen-activated protein kinase (MAPK) family is responsible for important signaling pathways. In this study, we report that ISAV activates ERK and p38 in Salmo salar. In salmonid macrophages, while ERK was required for SOD, GLURED, p47phox expression, p38 regulated the ROS production by the NADPH oxidase complex activation. These results, together with the presence of several consensus target motifs for p38 MAPK in the promoter of the S. salar p47phox gene, suggest that p38 MAPK regulates p47phox gene expression in fish through the activation of this key transcription factor.

122) Identification and biochemical characterization of a rhamnogalacturonan acetylesterase from *Penicillium purpurogenum* heterologously expressed in *Pichia pastoris*

Oleas, G[1]., Callegari, E[2].,Pizarro, M[1].,Sepulveda, R[1].,Gonzalez-Nilo, D[1].,Eyzaguirre, J[1].,[1]Ciencias Biologicas Universidad Andrés Bello.[2]Proteomics Facility Universidad de Dakota del Sur. (Sponsored by FONDECYT 110084 And 1130180, UNAB DI-478-14/R And DI-73-12/I And UNAB Scholarship To G.O.)

Rhamnogalacturonan acetylesterases (RAEs) catalyze the deacetylation of rhamnogalacturonan. *Penicillium purpurogenum* is a fungus which grows on sugar beet pulp (SBP) which is composed, in part, of esterified rhamnogalacturonan. The aim of this work is the heterologous expression of a RAE from *P. purpurogenum* and to characterize the recombinant enzyme. *P. purpurogenum* was grown on SBP. Partially purified culture supernatant was loaded on a zymogram. An area active on methylumbelliferyl-acetate (MUA) was analyzed by mass spectrometry. The peptides obtained matched a hypothetical RAE (RAEA). RAEA coding sequence was heterologously expressed in *Pichia pastoris.* The recombinant enzyme was purified. SDS-PAGE showed a molecular mass of 30kDa. RAEA is active toward: MUA, indoxyl acetate. fluorescein diacetate and p-nitrophenyl acetate (Km= 1.63 mM). It is not active toward p-nitrophenyl derivatives of ferulate, decanoate, palmitate and dodecanoate. Bioinformatic analysis predicts the typical catalytic triad of serine esterases (ser-his-asp). Distinctive motifs of the SGNH-hydrolase family were also detected. In conclusion, RAEA is a rhamnogalacturonan acetylesterase secreted by *Penicillium purpurogenum* during sugar beet pulp degradation. It belongs to the SGNH-hydrolases family. It is the second fungal RAEA biochemically characterized, thus highlighting the novelty of this work.

123) Expression and enzymatic activity evaluation of the DNA glycosylase NTH1 from *Trypanosoma cruzi*

Ormeño, F[1]., Barrientos, C[1].,Sepúlveda, S[1].,Ponce, I[1].,Valenzuela, L[1].,Campos, C[2].,Astorga, R[1].,Cabrera, G[1].,Galanti, N[1].,[1]Programa de Biología Celular y Molecular, ICBM, Facultad de Medicina, Universidad De Chile.[2]Programa de Farmacología Molecular y Clínica, ICBM, Facultad de Medicina, Universidad De Chile. (Funded By FONDECYT Project N° 1130113 (N.G.))

Trypanosoma cruzi, a parasitic protozoan, is the etiological agent of Chagas disease, an endemic pathology in Latin America. Currently, there are no effective drugs to treat this disease in chronic patients. There are evidences that the parasite is able to resist oxidative DNA damage at different stages of its life cycle. The Base Excision Repair System (BER) is one of the main DNA repair mechanisms in eukaryotes. DNA glycosylases are enzymes involved in the recognition of oxidative DNA damage, constituting the first step of the BER pathway. Among the DNA glycosylases, NTH1 is a bifunctional enzyme that removes pyrimidine oxidized derivatives, and then catalyzes the cleavage of the DNA strand through an AP lyase activity. The *T. cruzi* NTH1 enzyme (TcNTH1) was expressed and purified in *E. coli*, in order to generate polyclonal antibodies in mice and to perform enzymatic activity assays. With the antibodies obtained, TcNTH1 expression was identified in the three cellular forms of *T. cruzi*. Surprisingly, TcNTH1 does not remove the oxidized base thymine glycol (TG) present in a synthetic labeled oligonucleotide though it was found an apurinic-apyrimidinic activity of this enzyme. Therefore, TcNTH1 is the first DNA glycosylase described unable of catalyzing the cleavage of a TG substrate.

124) Adipopnectin and TNF-α signaling in endometria from obese women with polycystic ovarian syndrome (PCOS)

Oróstica, L[1]., Astorga, I[1].,García, V[2,1].,Poblete, C[1].,Romero, C[1,3].,Vega, M[1,3].,[1]Laboratory of Endocrinology and Reproductive Biology, Clinical Hospital, University of Chile.[2]School of Medicine University of Antofagasta.[3]Department of Obstetrics and Gynecology, Faculty of Medicine, University of Chile. (Sponsored by FONDECYT #1130053 (MV); CONICYT # 21120541 (LO))

Seventy percent of women bearing PCOS are obese; adiponectin and TNF-α, as obesity markers, have an important role in the sensitivity and action of insulin receptor. Adiponectin (insulin sensitizing) decreases, whereas, TNF-α (negative regulator of insulin pathway) increases in obese-women. These changes could affect the normal energetic status in endometrium, tissue that exhibits abnormal insulin signaling in the PCOS condition (hyperandrogenic/hyperinsulinic environment). The aim of the present work was to evaluate protein and transcripts levels of molecules involved in adiponectin (APPL1, MEK1, p38-MAPK) and TNF-α (RTNF-α 1 y 2, NFkB) signaling. These molecules were evaluated in endometria (n=15) from Lean, Obese and Obese-PCOS women by western-blot, immunohistochemistry and real-time PCR. Also, plasma levels of adiponectin and TNF-α were assayed by ELISA. Adiponectin protein levels (plasma and endometrial), APPL1 and MEK1 are low in Obese-PCOS vs Lean groups ($p<0.05$); p38-MAPK diminish in Obese endometria ($p<0.05$). TNF-α and its receptors increase in Obese-PCOS vs Lean endometria ($p<0.05$). Transcripts levels for adiponectin and TNF-α and their receptors and APPL1 were similar in the three groups of endometria. Therefore, in endometria from Obese-PCOS women, adiponectin and TNF-α pathways are deregulated, where obesity, hyperandrogenic and hyperinsulinic conditions may affect insulin signaling, thus, compromising the energetic metabolism for normal endometrial function.

125) Endocytic trafficking impacts vacuole structure affecting root architecture in *Arabidopsis thaliana*

Osorio, C[1]., Pizarro, L[1].,Norambuena , L[1].,[1]Centro Biología Molecular Vegetal , Facultad de Ciencias, Universidad De Chile. (Sponsored by FONDECYT 1120289)

The trafficking endocytic pathway is responsible for Plasma Membrane (PM) and extracellular components internalization. The final destination on the endocytic pathway is the vacuole which is involved in the components storage and degradation and cell growth. Our laboratory has characterized the transcription factor bZIP25 as a negative regulator of the endocytic pathway. Its mutant, *bzip25-2*, has accelerated endocytosis from PM to the vacuole while the over-expressor line OX-bZIP25 has the opposite phenotype. *bzip25-2* establishes a central large vacuole faster than the wild type (Wt), whereas OX-bZIP25 has multiple vacuoles of variable sizes. In *bzip25-2,* PM endocytosed membrane reaches the vacuole at earlier times than in Wt even in the presence of the endocytic inhibitors, Brefeldin A (BFA) and Wortmannin (Wm). Both drugs induce changes in vacuole structure in Wt plants. BFA-treated *bzip25-2* presents more elongated vacuoles than BFA-treated Wt, being actually closer to untreated Wt. Interestingly, *bzip25-2* root system has higher lateral root density and longer primary and lateral roots than the Wt in normal condition also under BFA-endocytic inhibition. In both conditions, *bzip25-2* and Wt vacuole structures are different. Furthermore, Wm causes the same alteration on vacuole structure which correlates with similar root architecture in both lines. Together these results link endocytic trafficking modulation, vacuole structure and root system growth dynamics.

126) Decrease in NAD^+ levels disrupts mitochondrial metabolism and adaptive response in cardiomyocytes.

Oyarzun, A[1].,Troncoso, R[1].,Pennanen, C[1].,Lavandero, S[1,2].,[1]Advanced Center for Chronic Disease (ACCDiS), Facultad de Ciencias Quimicas y Farmaceuticas, Universidad De Chile.[2]Department of Internal Medicine, Southwestern Medical Center, University of Texas. (Sponsored by FONDAP 15130011 (SL And RT), FONDECYT 1120212 (SL), ACT1111 (SL), FONDECYT 3130749 (CP), FONDECYT 11130285 (RT))

NAD^+ is a coenzyme with multiple functions, including electron carrier in redox metabolism, energy sensor, substrate of deacetylases and Ca^{+2} mobilization. NAD^+ levels are important in cardiovascular diseases progression, decreased NAD^+ levels have been reported in several cardiomyopathies and have been associated to cardiovascular risk. The aim of our work was to investigate the effects of NAD^+ decrease on energy metabolism and adaptive response in cardiomyocytes. We used the Nampt inhibitor FK866 to reduce NAD^+ levels in cultured rat cardiomyocytes. Cell viability, mitochondrial metabolism, and cardiomyocyte response to hydrogen peroxide (H_2O_2) and norepinephrine (NE) were assessed. The results showed that lowering NAD^+ levels: a) decreased mitochondrial metabolism without alteration on cell viability, b) diminished H_2O_2-dependent cardiomyocyte survival, and c) prevented the development of hypertrophy triggered by NE (cell area and sarcomerization). All these effects were prevented by recovering NAD^+levels with nicotinamide mononucleotide (NMN), excepting the hypertrophic response. This work reveals that NAD^+ is essential for cardiomyocyte mitochondrial metabolism and survival.

127) Cytokinin-dependent transcriptional regulation of PIN auxin efflux carriers

OBrien, J[1,2,3]., Simásková, M[2,3].,Cuesta, C[3,4,2].,De Clercq, I[2,3].,Van Brausegem, F[2,3].,Benkova, E[2,3,4].,[1]Department of Molecular Genetics & Microbiology , Faculty of Biological Ciences, Pontificia Universidad Catolica de Chile..[2]Department of Plant Biotechnology and Bioinformatics Ghent University.[3]Department of Plant Systems Biology VIB.[4]. Institute of Science and Technology Austria.

During plant development, the interaction between auxin and cytokinin is essential for the modulation of plant architecture. Cell to cell transport of auxin by PIN-FORMED auxin efflux carriers (PINs) is key to achieve an auxin maxima. This will allow the proper maintenance of the meristems and the formation of new organs, such as lateral roots. However, the positive effect of auxin is antagonistically regulated by cytokinin. It has been shown that cytokinin regulates the expression of several *PINs* at a transcriptional level. Interestingly, in response to cytokinin *PIN1* is down-regulated and *PIN7* is up-regulated. Due to their opposite cytokinin-dependent regulation, both *PIN1* and *PIN7* were selected to elucidate the transcription network that regulate them in a cytokinin-dependent manner. In both promoters, we revealed the regulatory elements necessary for the cytokinin response. In order to find the upstream regulators mediating cytokinin control over PINs, a yeast-one-hybrid screen was performed and several transcription factors were identified. Phenotypic characterization of Arabidopsis mutants for the selected transcription factors show an impaired response to cytokinin treatment.

128) Acute administration of corticosterone to adrenalectomized rats changes miRNAs levels in the hippocampus: relationship with AMPA and NMDA receptors expression

Pacheco, A[1]., Muñoz-LLanos, M[1].,García-Rojo, G[1].,Aguayo, F[1].,García-Pérez, M[1].,Márquez, R[1].,Werner, M[1].,Cidlowski, J[2].,Fiedler, J[1].,[1]Bioquímica y Biología Molecular, Ciencias Químicas y Farmacéuticas, Universidad de Chile.[2]Signal Transduction Laboratory Molecular Endocrinology Group NIEHS, USA. (Sponsored by FONDECYT 1120528)

The hippocampus is an important target for glucocorticoids (GCs) because of its high abundance of corticosteroid receptors that modulate glutamatergic neurotransmission, changing NMDA and AMPA receptor levels. However, it is unknown whether GCs regulate AMPA and NMDA receptor levels through miRNAs in the hippocampus. The aim of this work is to examine in rats with low levels of corticoids (adrenalectomized, ADX) if an acute corticosterone (CORT) administration, promotes changes in miRNA levels that target AMPA and NMDA receptor subunit mRNAs in the hippocampus. For this purpose, adrenalectomized male Sprague-Dawley rats were injected with CORT (10 mg/kg i.p.) and sacrificed at different times (0.5; 2.5; 6; and 24 h) post-administration. The hippocampi were processed for RNA and protein obtention and detection. After 0.5 h of CORT administration, a rise in mRNA levels of immediate early genes as Arc (2-fold) and c-Fos (2.5-fold) was observed. Interestingly, both mRNAs are related to an increased glutamatergic neurotransmission, suggesting an enhanced excitatory neurotransmission. Moreover, after 6 h of CORT administration we observed an increase (3-fold) in GILZ mRNA, a specific marker for glucocorticoid action. Using a miRNA microarray assay with samples obtained after 6 h of CORT administration, we found that miR-223 increases (1.7-fold) in contrast to the reduction in miR-3596a (4.8-fold), both being target miRNAs of GluA2 AMPA subunit and NR2A NMDA subunit.

129) Regulation and function of NUAK1 kinase during metabolic stress

Palma, M[1]., Riffo, E[1].,Pincheira, R[1].,Castro, A[1].,[1]Laboratorio de Transducción de señales y Cáncer, Departamento de Bioquímica y Biología Molecular, Ciencias Biológicas , Universidad De Concepción. (Sponsored by FONDECYT 1120923)

Cancer cells confronted to metabolic stress evade cell death during tumor progression. However, the underlying molecular mechanisms regulating the metabolic stress response are still unclear. In this study, we have investigated the regulation and function of the NUAK1 kinase on the cell survival response to metabolic stress. We evaluated NUAK1 protein and mRNA levels, NUAK1 protein stability, and NUAK1 role in the cell response to metabolic stress in immortalized mouse embryo fibroblasts (MEFs). We found that metabolic stress increases NUAK1 protein levels, through an increase in *NUAK1* gene transcription and protein stability. Furthermore, we determined that endogenous NUAK1 is phosphorylated, and promotes cell survival. Additional experiments suggested that phosphorylation of NUAK1 is involved in its functional response associated to metabolic stress. Overall, these results suggest that the cellular stress context is responsible for transcriptional and post-translational regulation of NUAK1, an effect that could be directly related to its cell survival function.

130) Herp protects Hela cells from oxidative stress-induced death by regulating the IP3 receptor

Paredes, F[1]., Gatica , D[1].,Troncoso, R[1].,Quiroga, C[1].,Parra, V[1].,Bravo, R[1].,Torrealva, N[1].,Navarro, M[1].,Pennanen , C[1].,San Martin, A[2].,Jaimovich, E[3].,Lavandero, S[1].,[1]Bioquímica y Biologia molecular, Ciencias Quimicas y Farmaceuticas, Universidad De Chile.[2]Cardiology, School of Medicine, Emory University .[3]Fisiologia, Medicina, Universidad De Chile. (Sponsored by ACT 1111 (SL, EJ), FONDAP 15130011 (SL), 3120220 (CQ), 3110114 (RT). FP, RB, NT And MN Hold CONICYT PhD Fellowships.)

Herp, an endoplasmic reticulum (ER) protein expressed in response to ER stress, osmotic stress or deregulation of Ca^{2+}homeostasis, exerts cytoprotective effects. It is not well understood whether Herp is also regulated by oxidative stress. We study here this problem and the stress-dependent signaling pathways in HeLa cells knockdown for Herp (Sh-Herp) or controls (Sh-Luc). Our results showed that protein levels of Herp increased in response to oxidative stress in HeLa cells treated for 30 min with H_2O_2 or with angiotensin II. Increased Herp levels were also observed in aorta cells isolated from mice treated with angiotensin II for 3 weeks. Cell death assessed by PI and flow cytometry was significantly higher in Sh-Herp HeLa cells treated with H_2O_2 in comparison with Sh-Luc HeLa cells. This effect was abolished by the intracellular Ca^{2+} chelator BAPTA-AM or the IP3 receptor (IP3R) antagonist xestospongin B (XeB), suggesting that this effect was dependent of intracellular Ca^{2+} stores and the IP3R. Intracellular Ca^{2+} kinetics shows that Sh-Herp HeLa cells exhibited a greater and more sustained Ca^{2+} increase over time that Sh-Luc HeLa cells. Similar results were observed in mitochondrial Ca^{2+} kinetics. This higher sensitivity to H_2O_2 of Sh-Herp cells was abolished when cells are pretreated with the opening MPTP inhibitor cyclosporine.These data indicate that Herp has a cytoprotective effect against oxidative stress by regulating the IP3R and the influx of calcium into mitochondria.

131) Disruption of mitochondria– ER communication triggers cardiomyocyte hypertrophy

Pennanen, C[1,3]., Troncoso, R[3].,Gutierrez, T[3].,Parra, V[2].,Jaimovich, E[1].,Lavandero, S[3,2].,[1]Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad De Chile.[2]Southwestern Medical Center, Dallas., UT.[3]Advanced Center for Chronic Diseases (ACCDiS), Faculty Chemical & Pharmaceutical Sciences & Faculty of Medicine, University of Chile.

The interaction between mitochondria and endoplasmic reticulum (ER) is a key regulator in Ca^{2+} homeostasis. In cardiomyocytes, cytosolic Ca^{2+} engages several signaling pathways related to hypertrophy such as calcineurin, CaMK and PKC. In this work, mitochondria-ER communication was disrupted in cultured rat cardiomyocytes using an antisense adenovirus against mitofusin 2 (Mfn2, a protein found in mitochondria-ER contact sites). After adenoviral treatment, cells developed hypertrophy with increases in beta-myosin heavy chain levels, ANF mRNA levels and cell area. This response was dependent of mitochondrial Ca^{2+} import because was mimicked using a siRNA against mitochondrial Ca^{2+} uniporter (MCU) and associated to activation of Ca^{2+}/calcineurin signaling pathway. We concluded that disruption of mitochondria–ER communication triggers the development of cardiac hypertrophy. Supported by FONDAP 15130011 (SL), FONDECYT 1120212 (SL), FONDECYT 15010006 (EJ), FONDECYT 3130749 (CP).

132) Comparison of the biochemical properties of two α-L-arabinofuranosidases GH62 from *Aspergillus fumigatus* wmo.

Pérez-Lara, R[1]., Eyzaguirre, J[1].,[1]Ciencias Biologicas, , Universidad Andrés Bello. (Sponsored by FONDECYT 110084 And 1130180, UNAB DI-478-14/R And DI-73-12/I.)

We have identified two genes encoding possible α-L-arabinofuranosidases belonging to family GH62 in the genome of the fungus *Aspergillus fumigatus* wmo. The first gene, *abfI*, has 999 bp and encodes a protein of 332 amino acids. The second gene, *abfII,* consists of 1246 bp including an intron of 59 bp, encoding a protein of 396 amino acids that contains a catalytic module and a carbohydrate binding module belonging to family CBM1. The predicted catalytic modules of both enzymes share a 79.37% identity. To compare their biochemical properties, both were heterologously expressed in *Pichia pastoris*. The optimum pH of ABFI is in the range of 4.0-5.0 and the optimum temperature is 37 °C, while the optimum pH of ABFII is in the range of 4.5-5.5 and its optimum temperature is 42 °C, being more thermostable than its paralogue. Both enzymes exhibit activity against p-nitrophenyl alpha-L-arabinofuranoside, with a Km of 3.9 mM for ABFII and a Km of 94 mM for ABFI. Both ABFII and ABFI are capable of releasing arabinose from rye and wheat arabinoxylan, being more active on the first. ABFII releases arabinose from arabinan, being the third GH62 enzyme exhibiting this activity. The presence of a CBM gives ABFII more stability and activity on a wider range of substrates as compared to ABFI.

133) Biochemical and cellular alterations in podocyte cells mediated by adenosine A_{2B} receptor

Pérez, G[1]., Jaramillo, C[1].,Alarcón, S[1].,San Martín, R[1].,[1]Instituto de Bioquímica y Microbiologia, Facultad de Ciencias, Universidad Austral de Chile. (Funded By Grant FONDECYT 1130414 And CONICYT 24121270)

Introduction. Podocyte cells dysfunction is a remarkable feature of diabetic glomerulopathy leading to the deterioration of the filtration barrier and proteinuria. Interestingly, the adenosine nucleoside and its A_{2B} receptor subtype locally increase during the progression of the diabetic glomerulopathy. Our aim will be to determine the role of adenosine in mediating podocyte cells dysfunction. *Results.* Exposure of rat podocytes to adenosine leads to actin redistribution, changes in cellular morphology, decrease expression of the integrin α3 adhesion molecule and induction of α-SMA, an epithelial to mesenchymal transition marker. Further, these changes appeared along with an increased migration rate. The above effects were blocked by an adenosine A_{2B} receptor subtype antagonist. Moreover, proteinuria was blocked by the A_{2B} receptor antagonist in diabetic rats. The glomerular induction of adenosine A_{2B} receptor could be mediated by the cytokine TNF-α and adenosine itself. *Conclusion*: Podocytes function losscould be triggered by increased levels of adenosine such as in diabetic nephropathy. This effect is relevant because affects the integrity of the glomerular filtration barrier. Furthermore, *in vivo* intervention using an adenosine A_{2B} receptor antagonistameliorates the proteinuria in diabetic animals.

134) Differential microRNA expression in breast cancer cell lines related to the presence or absence of BRCA1

Pérez, E[1]., Zavala, V[1].,Álvarez, C[1].,Carvallo, M[1].,[1]Biología celular y molecular, Ciencias Biológicas, Pontificia Universidad Católica De Chile. (Sponsored by FONDECYT 1120200)

microRNAs are small non coding RNAs that negatively regulate the expression of target genes at a post transcriptional level. Abnormal expression of different microRNAs is associated with the development of diverse cancer types; and several studies have shown differential expression of microRNAs between normal and tumor tissues. Knowing the different patterns of expression of these small RNAs in cancer, could be a helpful diagnosis and prognostic tool in the clinic. The aim of this study is to characterize the global expression of microRNAs, for two breast cancer cell lines (T47D and HCC1937) and one non tumor breast cell line (MCF10A) by using microarrays. The analysis showed 8 microRNAs differentially expressed in the cancer cell lines compared to the non tumor cell line: 5 were upregulated and 3 downregulated. Searching for the pathways in which these microRNAs are involved, we found that three of them participate in different cancer pathways. Two of these cell lines (T47D and MCF10A) express the wild type BRCA1 protein, which participates in the biogenesis of microRNAs via interaction with the DROSHA microprocessor complex. Comparing the cell lines T47D and MCF10A with HCC1937 (which express a mutant/truncated BRCA1) we found a group close to 150 microRNAs that were downregulated in the HCC1937 cells. This result suggests that the lack of BRCA1 in HCC1937 could be exerting a negative effect in the expression of these miRNAs. FONDECYT 1120200.

135) Effect of proteasome inhibition on the cardiac ischemia/reperfusion injury

Pino, G[1]., Pedrozo, Z[1].,Montecinos, L[1].,Donoso, P[1].,Lavandero, S[2].,Sánchez, G[3].,[1]Fisiología y Biofísica, Medicina, Universidad De Chile.[2]Bioquímica y Biología Molecular, Ciencias Químicas y Farmacéuticas, Universidad de Chile.[3]Fisiopatolgía, Medicina, Universidad de Chile. (Funded By Fondecyt 110257, 1130407, Programa U-Inicia And FONDAP 15130011)

Cardiac ischemia/reperfusion (IR) causes extensive protein degradation, contractile dysfunction and cell death. Inhibition of the proteasome has been used as a strategy to decrease IR injury, however beneficial and detrimental effects have been reported. We hypothesize that the proteasome is a key element of IR injury and its inhibition prevent the IR damage. In the *ex vivo* model (Langendorff perfused rat heart model) we determine that two proteasome inhibitors, MG132 and MLN-9708 reduced infarct size by 60% at low concentrations (up to 0.1 µM and 0.5 µM respectively) during IR (30 min global ischemia / 60 min reperfusion), but did not reduce the damage when concentrations of inhibitors were tenfold higher. Further, our data from simulated IR (sIR, 8 h ischemia / 16 h reperfusion) suggest that MG132 (4 µM) prevents the death of neonatal rat cardiomyocytes (*in vitro*). Autophagy was activated during sIR as evidenced by the increase in LC3-II and Beclin-1 and was even more active when cardiomyocytes were treated with MG132. On the other hand, we found a significant decrease in mitochondrial DNA content (50%) and mitofusin-2 (50%) protein levels during sIR but this decrease that was blunted in presence of MG132. These results suggest that low concentrations of MG132 or MLN9708 protect the heart from IR injury, partially through the effect on the mitochondrial content and mitofusin-2 protein levels.

136) Doxorubicin inhibits autophagy in cultured rat cardiomyocytes.

Pizarro, M[1]., Chiong, M[1].,Lavandero, S[1].,Lavandero, S[2].,[1]Advanced Center for Chronic Diseases (ACCDiS) and Center for Molecular Studies of the Cell (CEMC), Faculty of Chemical and Pharmaceutical Sciences & Faculty. of Medicine, Universidad De Chile.[2]Department of Internal Medicine, Southwestern Medical Center, University of Texas. (Supported By Grants Anillo ACT1111 (to SL), FONDAP 15130011 (to SL), CONICYT PhD Fellowship (to MP))

Doxorubicin (DOX) is an effective drug used for the treatment of a variety of cancer. However its use is limited by cardiotoxicity. The mechanism underlying DOX-induced cardiotoxicity is not completely understood, but oxidative stress seems to be involved at least in part, in this deleterious effect. More recently, the dysregulation of autophagy has emerged as another toxic mechanism. Autophagy is a vital process in the heart, participating in the removal of dysfunctional components and serving as a catabolic energy source during time of starvation.Autophagy serves to protect cells and may also contribute to cell damage. In this study, we evaluated the long-term effects of DOX on cardiomyocyte autophagy and its relationship to cell viability. Cultured rat cardiomyocytes were stimulated with DOX 1 uM for 24 h and autophagy markers LC3-II and p62 were analyzed in condition of autophagy flux. The results showed that DOX treatment inhibits autophagy. The LC3-II and p62 levels were also diminished even in presence of Bafilomicyn A (Baf A). Stimulation of DOX for 24 h also increased the percentage of cell death compared to untreated cardiomyocytes. We also found that DOX increased AKT, mTOR and p70S6K phosphorylation, one of the pathways that inhibites autophagy. We conclude that the inhibition of autophagy by DOX is associated to cardiomyocyte death.

137) The IGF-1/Calcineurin/MEF2 signaling pathway participates in the regulation of myostatin gene expression.

Quezada, A[1]., Fuentes, E[2].,Molina, A[2].,Valdés, J[1].,[1]Laboratorio de Bioquímica Celular, Facultad de Ciencias Biológicas. Interdisciplinary Center for Aquaculture Research (INCAR), Universidad Andrés Bello.[2]Laboratorio de Biotecnología Molecular, Facultad de Ciencias Biológicas. Interdisciplinary Center for Aquaculture Research (INCAR), Universidad Andrés Bello. (Sponsored by CONICYT/FONDAP/15110027)

Introduction: Skeletal muscle growth is regulated by a variety of growth factors, most notably IGF-1 and myostatin. IGF-1 is a positive regulator in proliferation and differentiation of skeletal muscle cells, while myostatin is a negative regulator of skeletal muscle mass. IGF-1 exerts its functions by activating different signaling cascades that regulate the activity of promyogenic transcription factors like MEF2. Bioinformatics analysis of murine myostatin promoter shows several conserved regions for MEF2, suggesting that IGF-1 may induces myostatin expression by regulating MEF2. **Materials and Methods:** Primary culture of rat skeletal myoblast were treated with recombinant IGF-1 (10 nM) in the presence of pharmacological inhibitors of the IGF-1/Calcineurin signaling pathway, and examined the phosphorylation status of MEF2A isoform by western blot and the MEF2-dependent transcriptional activity by luciferase reporter vector assay. **Results:** IGF-1 decreased the phosphorylation of MEF2A as well as increased the transcriptional activation of MEF2 via IGF-1 Receptor/ PI3K/ PLC gamma/ Calcineurin signaling pathways. Additionally, we found a significant increase in mRNA contents of myostatin and its reporter activity after treatment with IGF-1. **Discussion:** This study demonstrated that IGF-1 decreases the phosphorylation status of MEF2A and increase its transcriptional-dependent activity, suggesting the up-regulation of myostatin expression.

138) Flavonols: molecules that playing a role in the assimetrical growth of radiata pine in response to inclination

Ramos, P[1]., Guajardo , J[1].,Moya-León, M[1].,Herrera, R[1].,[1]Instituto de Ciencias Biológicas Universidad De Talca. (This Project Was Supported By FONDECYT Projects 11121170, 1120635.)

Stem reorientation is a widely studied phenomenon in trees, but the molecular mechanism is still unknown. Flavonols have been reported as potent inhibitors of polar auxin transport and therefore their participation in the inclination response has been addressed. Genes involved in the biosynthesis of flavonols and metabolite contents were analyzed in radiata pine seedlings exposed to 45º inclination. Stems were cut in two different ways: sectioning stems into 3 segments (apical, medial and basal) or longitudinally dissected into upper and lower halves. Full-length sequences of genes involved in phenylpropanoids, and specifically, in flavonols biosynthesis pathway were isolated from radiata pine. The expression of chalcone synthase, flavanone 3–hydroxylase and flavonol synthase genes performed by qRT-PCR indicated that they were induced in response to stem inclination; higher expression levels were recorded at the basal zone and in the upper half of the stem. The reduction in abundance of auxin repressed protein transcripts at the lower half of inclined stems indicates auxin distribution towards it, supporting the role of auxins in the reorientation process. Quercetin accumulates in the upper half of stems avoiding auxin distribution. At the same time, the expression of flavonols biosynthesis genes increased in the upper side of responding stem. This indicates a concerted activation of genes that generates a misbalance in local auxin distribution that finally induces stem reorientation.

139) NEXOS CHILE-USA: Creating networks to promote Chile's scientific future

Ramos, M. P[1]., Larrondo, L[2].,[1]Genetics Albert Einstein College of Medicine.[2]Genetica Molecular y Microbiologia Pontificia Universidad Católica De Chile.

Nexos Chile-USA is a non-profit organization created by and for Chilean Scientists residing in the United States, aiming to establish networks to propel Chile's scientific development. In U.S.A. there is a large mass of Chilean scientists engaged in graduate studies and postdoctoral training, as well as serving academic and scientific duties in research institutions. Nexos mission is to 1) Facilitate scientific interactions by creating formal ties between scientists and institutions in the U.S. and Chile 2) Help to locate and generate opportunities for individuals intending to work or advance their training in the U.S. 3) Contribute to the discussion of public policies related to the scientific reinsertion of numerous professionals pursuing training abroad and 4) Facilitate a better understanding between the government and the scientific community. With that purpose in mind, Nexos Chile-USA organizes an annual meeting that since 2010 is the founding initiative in bringing together Chilean scientists. During the conference, participants can present their work among piers and discuss, with perspective, the current and future state of science in Chile. These meetings have also included important opinion leaders, as well as relevant players of main funding agencies such as *Iniciativa Científica Milenio* and FONDECYT. Nexos is becoming an important platform to facilitate scientific exchange between Chile and the United States. The next meeting will take place in UPENN, PA, on October 17-18, 2014 (www.nexoschileusa.org/nexos@nexoschileusa.org) MN-FISB NC120043

140) Involvement of cathepsin L in the anti-apoptotic respond to genotoxic stress in COLO320 colorectal cancer line.

Reyes, C[1]., Riquelme, O[2].,Bustamante, S[2].,Fonseca, A[2].,Gutierrez, S[2].,Castro, A[2].,Morin, V[2].,[1]Bioquimica, Farmacia, Universidad De Concepción.[2]Bioquimica, Ciencias Biologicas, Universidad De Concepción.

DNA damage by genotoxic stress provokes disturbance of cell homeostasis, triggering activation of autophagic and apoptotic mechanisms. These particular processes directly or indirectly require lysosomal proteases such as cathepsin B, D and L. So far the role of cathepsin D and B with respect to different stress conditions has been described. As for autophagy, both cathepsins degrade the material which is carried out by the autophagosome. On the other hand, these particular proteases related to apoptosis inductors are relocated and they activate proteins belonging to the apoptotic cascade. The function of cathepsin L is uncertain at this respect, so the presumption is that under genotoxic stress cathepsin L participates in protein activation related to apoptosis and in the degradation of the material which is carried by the autophagosome during autophagy. To obtain additional information a genotoxic stress was applied to colo320 colon cancer cells; an etopoxide compound was employed and DNA degradation was observed both by means of agarose gels fragmentation as well as by comet assay. On the other hand, cathepsin L location was analyzed by means of immune-cytochemistry; it was noted that under stress conditions with the etopoxide compound, cathepsin L shows some location loss with respect to its normal location in the lysosome. Western blot analysis shows an increase of cathepsin L and caspase-3 expressions; this was reverted when siRNA against this cathepsin L were employed. Grant: VRID-enlace 214.037.018-1.0, FONDECYT 1120923.

141) Developing an automated MMGBSA-based protocol to estimate binding free energies of protein-ligand complexes

Reyes, L[1]., Adasme-Carreño, F[2].,Alzate-Morales, J[2].,[1]Centro de Bioinformática y Simulación Molecular (CBSM), Escuela de Ingeniería en Bioinformática, Universidad De Talca. [2]Centro de Bioinformática y Simulación Molecular (CBSM), Facultad de Ingeniería, Universidad De Talca. (Sponsored by L.R. And J.A.M. Thank Project FONDECYT No. 1140618 And The School Of Ingeniería En Bioinformática, Universidad De Talca For The Granted Financial Support.)

Computer-aided drug design[1] have had a high relevance in the investigation of the interaction of small organic molecules with molecular targets of clinical interest, being the most fundamental goal the prediction of whether a given molecule will bind or not, and how strong, to an specific target. In this regard, the continuum solvent MM/GBSA method[2] has grown in popularity in recent years for its low computational cost, however there is still no general consensus on an efficient protocol that determines affinity energies in total agreement with experimental data. Our goal is to implement an automated protocol to estimate the binding free energiesfor a large set of molecular complexes of biological interest, includingexplicit energetic terms related to the protein and ligand deformation upon binding, and take advantage of recently reported applications that could contribute to a fast and robust scoring function. As preliminary results, we tested the developed protocol against some acetylcholinesterase inhibitors recently reported[3] obtaining good correlations with experimental data. References 1. Torre, P., et al. (2012). *Molecules* 17.10: 12072-12085. 2. Chen, C. Y. C. (2013). *Current topics in medicinal chemistry*, *13*(9), 965-988. 3. Srinivasan, J., et al. (1998).*Journal of the American Chemical Society*,*120* (37), 9401-9409.

142) Annotation, mapping and synteny analysis of coding sequences in *Xenopus laevis* and *Xenopus tropicalis*.

Riadi, G[1]., Ossandón, F[2].,Larraín, J[3].,Melo, F[4].,[1]Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile.[2]Center for Bioinformatics and Genome Biology, Fundación Ciencia & Vida, Universidad Andrés Bello.[3]Center for Aging and Regeneration and Millenium Nucleus in Regenerative Biology, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile.[4]Molecular Bioinformatics Laboratory, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile. (Sponsored By Fondecyt Project 3130441; Iniciativa Cientifica Milenio No. P07-011-F.)

Xenopus laevis and *Xenopus tropicalis* are two African clawed frogs used as main models in experiments of genetics and molecular biology to study embryonic development of vertebrates. Phenotypically they are very similar regardless the *X. laevis* whole genome duplication, respect to *X. tropicalis*. Their genomes are 1.7 and 3.1 Gbp long with 51,998 and 62,823 protein coding sequences respectively. A reciprocal mapping of their sequenced scaffolds could serve as a Rosetta stone for experiments from different areas. In this work, we have mapped Xenbase assembly versions *X. laevis* 7.1 and *X. tropicalis* 8.0 onto each other, annotated and mapped their protein coding sequences, and surveyed the synteny of a subset of orthologous genes. 72% of *X. tropicalis* contiguous 10 chromosomes and 86% of the *X. laevis* fragmented genome in the form of 943 scaffolds were coarse aligned in order to find the mapping between blocks of 5Kb in each species. Around 120Mbp from each genome consists in repeated blocks in the other species, and at least 480Mbp of inversions were estimated. Despite only 45% of average identity between their genome sequences, a little over 99.6% of the 3,475 orthologous genes studied are collinear or nearly collinear. 95% of the genes were found to be in the same corresponding genome position. The remaining 5% consists in intrascaffold gene rearrangements of one species respect to the other. Acknowledgements: Fondecyt project 3130441; Iniciativa Cientifica Milenio No. P07/011-F.

143) DNAviz - A new tool for visualization of protein-DNA interactions based on buried surface areas

Ribeiro, J[1]., Schüller, A[1,2].,Melo, F[1].,[1]Genética Molecular y Microbiología Pontificia Universidad Católica De Chile.[2]Molecular Bioinformatics Laboratory, Millennium Institute on Immunology and Immunotherapy, Pontificia Universidad Católica De Chile. (Sponsored by FONDECYT 1141172, 1131065 And ICM P09-016-F)

Protein-DNA interactions are pivotal to the regulation of genetic processes. Today close to 5000 protein-nucleic acid complexes were deposited in the Protein Data Bank and the number has grown 10-fold since the late nineties. Visualization of protein-DNA interactions in three-dimensional complexes can greatly facilitate the understanding of the nature of specific DNA recognition. Here, we propose a new visualization scheme which enables detection of surface areas involved in specific and unspecific recognition at a glance. We developed an easy-to-use PyMOL plugin dubbed DNAviz that highlights the interactions in a protein-DNA complex based on calculation of the buried surface area of the complex against the free protein and free DNA. Residues and nucleotides of the protein-DNA interface are painted in three colors representing interactions with the DNA backbone, bases or both. Color intensities correspond to the buried surface area in the complex. The plugin allows for the generation of publication quality images, all results can be written to text files, and a command line interface is provided for advanced users. The color patterns on the protein surface and DNA may be helpful to identify regions of DNA base and shape readout, and can be particularly useful in rapidly pinpointing the overall mode of interaction.

144) The Sall2 transcription factor promotes mouse embryo fibroblast cell migration

Riffo, E[1]., Castro, A[1].,Pincheira, R[1].,[1]Laboratorio de Transducción de señales y Cáncer, Departamento de Bioquímica y Biología Molecular, Ciencias Biológicas, Universidad De Concepción. (Sponsored by FONDECYT 1110821)

Sall2 is a poorly characterized transcription factor, member of the *Spalt* gene family involved in neurogenesis and differentiation. Interestingly, homozygous mutations in Sall2 have been linked to abnormalities in neural tube closure and to ocular coloboma, a congenital defect characterized by the lack of closing of the optical fissure during embryonic development of the eye. Both phenomena are related to defects in cell migration, suggesting that Sall2 could play a role in this process. By using immortalized Sall2-deficient and wild type Mouse Embryo Fibroblasts (MEFs), we investigated the role of Sall2 in cell migration. Wound healing and haptotaxis assays showed that Sall2 deficiency decreased the migratory capacity of MEFs. To investigate how Sall2 could promote cell migration, we searched for putative targets. Bioinformatic analysis identified that extracellular matrix metalloproteinases Mmp2 and Mmp9 gene promoters contain several Sall2 consensus binding sites. In addition, qPCR analysis of Mmp2 and Mmp9 indicated that their expression is dramatically decreased in Sall2-deficient cells supporting a relationship between Sall2, matrix metalloproteinases and cell migration.

145) Structural and evolutionary validation of a dimeric model of the respiratory syncytial virus (RSV) matrix protein.

Ríos-Vera, C[1]., Melo, F[1].,Schüller, A[1,2].,[1]Depto. Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile.[2]Molecular Bioinformatics Laboratory Millennium Institute on Immunology and Immunotherapy. (Sponsored by FONDECYT No. 1131065 And ICM P09-016-F)

Human respiratory syncytial virus (hRSV) is an enveloped RNA virus of the Pneumovirinae family and is the leading cause of pneumonia and bronchiolitis in infants and elders worldwide. The viral matrix protein M is central to virus assembly and budding. Oligomerization of M was shown to be pivotal to hRSV infectious virus production. Presently only a monomeric crystal structure of hRSV-M is available. We therefore generated a comparative protein structure model of an M dimer, based on the related human metapneumo virus (hMPV) matrix protein which was resolved as a dimer. We evaluated our hRSV-M dimer model with a custom version of the software EPPIC (Evolutionary Protein-Protein Interface Classifier) and compared various structural and evolutionary properties. We obtained a total interface area of 1711 Å2 for our hRSV-M model and 1533 Å2 for hMPV-M, and indentified a total of 10 fully buried core interface residues in hRSV-M and 8 in hMPV-M. The number of core residues at the dimeric-interface (>=95% buried) is a powerful discriminator of interface character. We further found that 56% of the core residues situated at the interface of hMPV-M align with the core residues of the interface of hRSV-M and 70% of these were completely evolutionary conserved. Our structural and evolutionary analysis indicated that our dimeric hRSV-M compares favorably with the hMPV crystal structure. These results might help us to improve the understanding about how hRSV-M participates in protein-protein interactions involved in oligomerization.

146) 5-aza-2'-deoxycytidine treatment induces a decrease in expression of the *MLH1* gene and changes its histone acetylation pattern in HeLa cells.

Riquelme, A[1]., Sepúlveda, J[1].,Gutiérrez, S[1].,[1]Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad De Concepción. (Sponsored by FONDECYT 1130697)

Several types of cancer have modified epigenetic code, both at the histone modifications and DNA methylation level. 5-aza-2'-deoxycytidine (5-aza-dC), a DNA demethylating agent that is used as a cotreatment with chemotherapy, can induce the re-expression of tumor suppressor genes. *MLH1* gene, which encodes a protein involved in DNA repair, is silenced in several cancer cell types; but can be in some cases re-expresed by 5-aza-dC treatment. We hypothesize that a global DNA demethylation, induced by treatment with 5-aza-dC, leads to a change in the histone acetylation pattern in the regulatory region of the gene, thus causing an increase in *MLH1* expression. To test this hypothesis we evaluated changes in DNA methylation and histone acetylation pattern at the *MLH1* promoter in HeLa cells treated with 5-aza-dC, through bisulfite genomic sequentiation (Bis-Seq) and chromatin immunoprecipitation (ChIP) respectively. Surprisingly, our results show that *MLH1* gene expression decreased in response to 5-aza-dC treatment. Moreover, *MLH1* promoter region is constitutively demethylated, and 5-aza-dC treatment results in increased histone H3 acetylation.

147) Involvement of cathepsin L in the anti apoptotic response to metabolic stress in the colorectal cancer line COLO320

Riquelme, O[1]., Reyes , C[1].,Bustamante, S[1].,Celis, M[1].,Fernandez, F[1].,Castro, A[1].,Morin, V[1].,[1]Departamento de Bioquimica y Biologia Molecular, Facultad de Ciencias Biológicas, Universidad De Concepción. (Sponsored by VRID-Enlace 214.037.018-1.0, Fondecyt 1120923.)

Tumor cells that are not irrigated with blood present metabolic stress due to lack of nutrients. However, these cells survive these unfavorable conditions through evasion of apoptosis or promotion of autophagy. Some lysosomal proteases such as cathepsin L, lose this location when subjected to conditions of stress, and participate in anti-apoptotic processes in osteosarcoma cells. However, it is unknown whether cathepsin L has the same function in colorectal cancer cells in which it is known to participate in cell proliferation. Based on these antecedents, we hypothesized that cathepsin L is involved in the anti-apoptotic processes in the colorectal cancer line COLO320, and thereby promotes their survival under metabolic stress. Thus, the cells were subjected to stress with serum-free medium without glucose and localization of cathepsin L respect to its normal lysosome location was subsequently determined by immunocytochemistry. Later, cell survival was measured by a cell viability assay using the MTT technique. Finally, apoptosis was measured by immunodetection of caspase 3 and DNA degradation by agarose gel analysis, both in treated and untreated cells with a specific inhibitor of cathepsin L. The results suggest that cathepsin L is necessary for the evasion of apoptosis and thus the survival of the cell line under study.

148) Nicotinamide effect on the differentiation of C2C12 cells to osteoblasts

Rivas, F[1]., Quevedo, E[2].,Gutierrez, S[1].,[1]Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad De Concepción.[2]Departamento de Medicina interna, Facultad de Medicina, Universidad De Concepción. (Sponsored by FONDECYT Nº 1130697)

Osteoblasts are responsible for generating the matrix of bone tissue, these mesenchymal stem cells respond to various growth factors such as BMP-2, which activates the differentiation program. C2C12 cells can differentiate into muscle or bone cells; because of its bipotenciality are used as a model for studies of cell differentiation. It has been reported that a single mutation in the BMP type I receptor-2 (ACVR1) produces its constitutive activation which gives rise to the condition known as Fibrodysplasia ossificans progressive (FOP), characterized by the progressive formation of bone in extraskeletal sites. A new treatment based on the administration of nicotinamide in patients with this disease has slowdown the ectopic calcification. However, little is known about the effect that nicotinamide may have on osteoblast differentiation, and if so, what are the genes affected by the treatment. Therefore, we evaluated the effect of nicotinamide on gene expression and C2C12 BMP-induced differentiation into osteoblasts. Our results show that nicotinamide inhibit osteoblast differentiation induced by BMP-2 in a dose dependent manner and this treatment also reduces the expression of bone-related genes.

149) Rate limiting steps for folding and threading mechanism of MJ0366 from *Methanocaldococcus jannaschii*, a small knotted protein.

Rivera, A[1]., Reyes, J[1].,Marqusee, S[2].,Baez, M[1].,[1]Departamento de Bioquímica y Biología Molecular, Laboratorio de Bioquímica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.[2]California Institute for Quantitative Biosciences and Department of Molecular and Cell Biology University of California Berkeley. (Sponsored by FONDECYT 11110534)

In general, protein folding is guided by the formation of native contacts and obstructed by non-native ones which can lead to misfolded conformations or intermediates. However, knotted proteins are forced to form non-native contacts since their polypeptide chain must be threaded to reach the native state. MJ0366 from *M. jannaschii* has a trefoil knot which folding mechanism has been widely studied *in silico* to determine how non-native contacts guide the threading. There is no experimental data supporting these studies. In solution MJ0366 was a dimer as indicated by size exclusion chromatography measurements. Equilibrium stability curves, followed by circular dichroism, showed two state unfolding mechanism characterized by a single transition which middle point was independent of protein concentration supporting that subunit stability is uncoupled from the intersubunit contacts. Kinetic traces of unfolding and refolding reactions were well described by a single exponential function without apparent burst phases. Nevertheless, the dependence of kinetic constants with concentration of guanidine chloride showed a deviation from the linearity expected for a two-state system. These results are in agreement with an on-pathway intermediate before the threading step predicted by *in silico* studies. Experiments are underway to determine if threading of the polypeptide chain occurs before or after the metastable intermediate founded in this work.

150) Characterization of the ADP-Ribosylation mark on cytosolic histones H3 and H4

Rivera, C[1]., Vale, C[1].,Loyola, A[1].,[1]Epigenetics & Chromatin Laboratory Fundación Ciencia & Vida. (This Work Is Funded By FONDECYT 1120170, And Basal Project PFB16. Carlos Rivera Was Supported By CONICYT Master's Fellowship 22121806.)

ADP-ribosylation of histones has been classically studied in the regulation of DNA repair. However, this modification has been recently detected in the cytosolic pool of histones H3 and H4, suggesting a possible role in the histone post-translational processing before its nuclear translocation. This novel ADP ribosylation mark is a transient modification that can only be detected on the first two complexes that belong to the cascade of histone processing occurring after their synthesis. Therefore, our hypothesis is that ADP-ribosylation affects the folding state of newly synthesized H3 and H4 polypeptides. It is unknown which histone residues are modified and which are the enzymes that impose these marks. Thus, in the present study we performed chemical stability assays to investigate the acceptor amino acid. In addition, we addressed knock down experiments of putative cytosolic ADP Ribosyl Transferases to identify a responsible enzyme for this mark. We will discuss our recent findings.

151) Anti-atrophic effect of angiotensin (1-7) in skeletal muscle is enhanced by the use of dendrimers

Rivera, J[1]., Morales, G[1].,Abrigo, J[1].,Pacheco, N[2].,Márquez, V[2,3].,Ratjen, L[2,3].,Araya, I[2,3].,Gonzalez-Nilo, F[2,3].,Cabello-Verrugio, C[1].,[1]Laboratorio de Biología y Fisiopatología Moelcular, Depto de Ciencias Biológicas, Facultad de Ciencias Biológicas, Universidad Andrés Bello.[2]Center for Bioinformatics and Integrative Biology (CBIB), Depto de Ciencias Biológicas, Facultad de Ciencias Biológicas, Universidad Andrés Bello.[3]Fraunhofer Chile Research .. (Sponsored by JCR And VM Thank CONICYT Doctoral Fellowship. Support: FONDECYT 1120380, 3130593, Association-Francaise Contre Les Myopathies AFM 16670, UNAB DI-280, Fraunhofer Chile Research, Innova-Chile CORFO (FCR-CSB 09CEII-6991) And Anillo Científico ACT1107.)

Angiotensin (1-7) (Ang1-7), the main peptide of non-classical renin-angiotensin system, has beneficial effects in several tissues including skeletal muscle. We recently demonstrated that Ang1-7 has anti-atrophic effect on skeletal muscle increasing AKT phosphorylation and counteracting the effect of atrophic factors angiotensin-II and myostatin. Since Ang1-7 has a short half-life, is necessary the development of new methods of delivery to improve its bioavailability. Dendrimers, such as PAMAM–XX, are promissory vehicles to protect and transport bioactive molecules. We evaluated the effects of the new complex Ang1-7/PAMAM–XX dendrimer in the skeletal muscle atrophy in vitro and in vivo. In C2C12 myotubes, Ang1-7/PAMAM–XX increases AKT phosphorylation compared to Ang1-7 alone. Also, this complex shows a major diminution of muscle atrophy induced by myostatin and angiotensin-II. In a model of immobilization-induced atrophy in mice, Ang1-7/PAMAM–XX presents a higher recovery of muscle strength and fiber diameter, showing an improved anti-atrophic activity relative to Ang1-7 alone. In summary, our results show that the PAMAM–XX dendrimer improves the anti-atrophic activity cof Ang (1-7) on the skeletal muscle.

152) Development and characterization of a proviral-based reporter vector to study the role of the viral protein Rev in the control of HIV-1 gene expression.

Rojas, B[1]., Soto-Rifo, R[1].,[1]Programa Virología, ICBM, Medicina, Universidad De Chile. (Sponsored by FONDECYT 11121339)

The HIV-1 genomic RNA (gRNA) plays at least three major roles during viral replication as it i) undergoes alternative splicing to generate the 2-kb fully spliced and the 4-kb partially spliced transcripts; ii) is used as an mRNA for the synthesis of Gag and Gag-Pol precursors and iii) serves as the genome incorporated into progeny virions. While nuclear export of the 2-kb fully spliced viral transcripts follows the classical cellular mRNA export pathway mediated by NXF1, intron-containing transcripts such as the gRNA may overcome quality control mechanisms that normally retain and degrade intron-containing transcripts within the cell nucleus. However, the viral protein Rev acts as an adaptor between the gRNA and the host export factor CRM1, thus ensuring the nuclear export of this class of viral transcripts. In addition, it has been suggested that Rev could also promote viral mRNA translation by an unknown mechanism. In order to evaluate the role of Rev in HIV-1 gene expression, we developed and characterized a proviral-based reporter DNA carrying a mutation that abolished Rev expression. This mutation resulted in a strong inhibition of Gag expression from the gRNA that was rescued by expression of Rev in trans. Analysis of the molecular mechanisms at play confirmed the role of Rev in nuclear export and revealed a critical role on gRNA translation. Interestingly, this activity of Rev is partially exerted by acting at the 5´-UTR. The role of Rev in coupling gRNA nuclear export and its association with the host translational machinery will be discussed.

153) Relationship between secondary structure and antifreeze activity for hydroxiproline and proline homopeptides.

Rojas, R*[1,2]., Guzmán, F[1,3].,Aróstica, M[1,2].,Ojeda, C[1,3].,Marshall, H[4].,Carvajal-Rondanelli , P[5].,[1]Núcleo de Biotecnología Curauma Pontificia Universidad Católica De Valparaíso.[2]Instituto de quimica Pontificia Universidad Católica De Valparaíso.[3] Research Foundation Fraunhofer Chile.[4]Instituto de Biologia Pontificia Universidad Católica De Valparaíso.[5]Escuela de Alimentos Pontificia Universidad Católica De Valparaíso. (Work Funded By Grant 1140926 From FONDECYT, Chile. * Beneficiario Beca Postgrado PUCV 2014.)

Cryopreservation of cells, tissues and organs by using antifreeze peptides (AFP) as potential cryoprotectants is of great interest in areas of biotechnology, plant and animal breeding and medicine. Previous studies have shown that some natural AFPs adopt polyproline type II (PPII) secondary structure. To study the relationship between secondary structure and antifreeze activity, hydroxyproline (Hyp) and proline (Pro) were used as homopeptide models, considering that both types of peptides consist mainly of PPII secondary structure. For this work, both Pro and Hyp homopeptides between 10 and 14 residues were synthesized by solid phase peptide synthesis using F-moc methodology. The PPII structure of these peptides, determined by circular dichroism spectroscopy, was enhanced by increasing the number of residues, and also favored by decreasing the temperature. The antifreeze activity results, measured by differential scanning calorimetry, showed that the inhibition of ice recrystallization mediated by these homopeptides is also correlated with the propensity to adopt PPII structure and the number of amino acid residues of the peptide. Comparing the two sets of homopeptides, Hyp homopeptides showed a much stronger definition of its secondary structure and higher antifreeze activity, indicating that the addition of a hydroxyl group in proline residues strongly favored structure stabilization and recrystallization inhibition in both magnitude and ice crystal morphology.

154) Phospholipase D of the venom of *Loxosceles laeta* spider, induces the expression of inflammatory cytokines and chemokines on human skin fibroblasts.

Rojas, J., Aran, T.,Jaldin, R[1].,Cortes , E[1].,Araya, J[1].,Catalán, A[1].,[1]Tecnología Medica, Facultad de Ciencias de la Salud , Universidad De Antofagasta. (Sponsored by FONDECYT INICIACION 2013 Nº1113020)

The family of phospholipases D (PLD) of the venom of *Loxosceles's* spiderscan hydrolyze sphingomyelin to form choline and ceramide-1-phosphate (C1P). Together, they are capable of hydrolyzing lysophosphatidylcholine to form choline and lysophosphatidic acid (LPA). The role of C1P in triggering the inflammatory event observed in loxoscelism is unknown. C1P is produced in an intracellular pathway by the action of a ceramide kinase (CerK) or an extracellular pathway less common receptor dependent. Also, C1P induces proliferation, migration and pro-inflammatory action on macrophages involving a receptor-depending extracellular pathway. The aim was to evaluate the profile of inflammatory cytokines and chemokines on HFF-1 human skin fibroblast treated with venom of *L. laeta* and *Loxosceles* phospholipase D. The production profile of cytokine and chemokine inflammatory was evaluated by ELISArray in human skin fibroblast HFF-1 cell line treated with venom of *L. laeta*, r*Ll*PLD1, C1P and LPA. Also, the expression profile was evaluated by RT-PCR. None of the treatments showed a cytotoxic effect on fibloblasts. Also, IL-8 was the principal inflammatory cytokine produced by fibroblasts treated, minor production was observed for IL-6. CXCL1 and CXCL2 was the principal chemokines produced when fibroblasts was treated with venom *L. laeta* and rLlPLDs. Our data, allow us to guide on the triggering mechanism for the inflammatory reaction mediated by the venom of *Loxosceles* spiders.

155) Mouse liver agmatinase support polyamine biosynthesis *in vivo.*

Romero, N., Moreira, C[1].,Benítez, J[1].,Carvajal, N[1].,Uribe, E[1].,[1]Bioquímica y Biología Molecular, Ciencias Biológicas, Universidad De Concepción.

Agmatine plays important biological actions as a neurotransmitter, precursor for polyamines biosynthesis, hypoglycemic factor and many others. One central question is, therefore, how the cellular levels of agmatine are regulated. Agmatinase is involved in agmatine degradation (to putrescine and urea) and, although the properties of the bacterial enzyme are well known, the information about the mammalian enzyme is very scarce. Sequence alignments of agmatinases from different species, shows that mouse species differs in four of the seven key residues involved in binding of the essential Mn^{2+} ions. It was, therefore, proposed that the mouse enzyme should be unable to catalyze the hydrolysis of agmatine. In this study, we have expressed a mouse liver agmatinase cDNA and evaluated the ability of the recombinant protein to support the synthesis of polyamines under *in vivo* conditions, using a *Saccharomyces cerevisiae* strain which is unable to perform this biosynthetic process. In the absence of exogenous polyamines, yeasts cells transfected with mouse agmatinase were able to grow in the presence of agmatine. We, therefore, conclude that the incorporated agmatinase provides the putrescine which is required by the defficient yeast cells for polyamine synthesis and, consequently, for growth. Future work will evaluate the interaction of the activating metal ion with this particular agmatinase. Fondecyt 1120663.

156) Prooxidant behavior of quercetin alters mice erythropoiesis and heart mitochondrial function

Ruiz, L[1]., Salazar, C[1].,Jensen, E[2].,Elorza, A[2,3].,[1]Centro de Investigación Biomédica, Facultad de Ciencias de la Salud, Universidad Autónoma De Chile.[2]Center for Biomedical Research Universidad Andres Bello.[3]Millennium Institute of Immunology and Immunotherapy Universidad Andrés Bello. (Sponsored by FONDECYT 3110171, 11130192, 1100995. IMII P09-016-F)

Quercetin, a dietary plant flavonoid used as a food supplement, chelates iron disturbing iron homeostasis. *In vitro* studies defineda prooxidant effect of quercetin and described an interaction with the mitochondrial membrane causing a decreased fluidity, $O_2^{\cdot -}$ production, decreased ATP levels and respiratory chain inhibition. Our work studied the effect of quercetin *in vivo*. Mice were IP injected with 50mg/Kg for 15 days. Erythropoiesis and Heart mitochondrial function were analyzed. Quercetin decreased body weight, serum insulin and ceruloplasmin. Ferritin was not altered. Along with a decreased plasma total antioxidant capacity a significant delay on erythropoiesis progression was observed.Heart mitochondria displayed more protein oxidation and less activity of complex I and IV. In addition, a significant decrease of MFN2 and porin proteins was observed. All these results are in agreement with a prooxidant capacity of quercetin. This work contributes to the understanding of the mechanism of action of quercetin and suggest that its potential use in healthy people as a dietary supplement should be reexamined.

157) Expression analysis of *HATs* and *HDACs* genes in tomatoes under drought stress.

López-Barreaux, C[1]., Ruiz-Lara, S[1].,[1]Instituto de Ciencias Biológicas Universidad de Talca. (Sponsored by FONDEF D08I1118)

Several studies have related the stress response gene expression with chromatin remodeling, indicating that exists an additional step, upstream to known, regulating gene expression. Also, it has been observed in *Arabidopsis* and rice, that some enzymes that modify histone´s tails could be regulated by ABA and abiotic stress, participating in chromatin remodeling triggered by stress. The histone tail modification most studied is the acetylation, carried out by HATs enzymes, causing chromatin relaxing and allowing transcription. In contrast, HDACs, remove acetyl groups, causing chromatin compressing and inactivation of transcription. To understand the dynamics of chromatin remodeling under drought stress, we compare the transcriptional response of *HATs* and *HDACs* genes in two species of tomato genetically related *Solanum lycopersicum* and *S. chilense,* sensitive and drought tolerant, respectively. Both species were submitted to drought by stop watering and RNA total and cDNA were obtained from leaf samples. By mean of qRT-PCR, 9 *HATs* and 14 *HDACs* genes were analyzed. It was shown that exist transcriptional regulation triggered by drought in both kind of enzymes and some of them present differences between species. One finding inside this research was 2 HATs, *HAC1* and *HAC2* in *S. lycopersicum* showed a peak at 5th day of drought, in contrast, in *S. chilense*, were repressed by the treatment, showing a strong induction when plants were rehydrated.

158) Analysis of expression levels of *1-sst* and *6G-fft* genes responsible for the biosynthesis of fructans in *Aloe barbadensis* Miller plants undergoing drought conditions.

Salinas, C[1]., Caamaño, J[1,2].,Cardemil, L[1].,[1]Departamento de Biología, Facultad de Ciencias, Universidad De Chile.[2]Instituto de Química, Ciencias, Pontificia Universidad Católica de Valparíaso.

Aloe barbadensis Miller, also known as Aloe vera, is a CAM plant adapted to grow in arid environments. One of its adaptations is an efficient osmotic adjustment which results in the synthesis and accumulation of carbohydrates in its leaves. Fructans are a group of non-structural reserve polysaccharides that consists mainly of fructose units linked to a terminal glucose and that have been shown to protect the integrity and function of cell membranes and proteins. Previous results from our group have shown that Aloe plants grown under water stress have higher concentrations of neo-fructans in their leaves compared to plants cultivated in normal irrigation conditions. Since this variation can be due to an increased enzymatic activity and/or variations in the expression of certain genes involved in the metabolic pathway of fructans, hence we have focused to analyze the presence, sequence and expression of two important genes involved with fructan synthesis in Aloe vera. The key genes selected were *1-sst,* sucrose: sucrose 1-fructosyltransferase which is responsible for the synthesis of 1-kestose and *6G-fft,* fructan: fructan 6G-fructosyltransferase which synthesizes neo-kestose. Our preliminary results indicate the presence of both genes *1-sst* and *6G-fft* in Aloe vera plants. Further analyzes contemplated include qRT-PCR of mRNA for both fructan genes from Aloe plants and the possible influence of exogenous ABA in there expression levels. Acknowledgment: FONDECYT 1130025.

159) Increased level of adenosine and enzymatic activity of CD73 precedes renal dysfunction ib diabetic rats.

Salinas, P[1]., Gómez, D[1].,Oyarzún, C[1].,Jaramillo, C[1].,San Martín, R[1].,[1]Instituto de Bioquímica y Microbiología, Ciencias, Universidad Austral de Chile. (Financed By FONDECYT 1130414 And DID-UACH 2013-15)

Introduction: Diabetic nephropathy (DN) remains the leading cause of end-stage renal disease worldwide. The biochemical disorders related to the origin of this disease have not been clearly established. Interestingly, it has been demonstrated that the plasmatic adenosine is elevated in patients with DN. Furthermore, studies in experimental models show that signaling of this nucleoside can generate renal cell dysfunction. This study aims to correlate the progression of diabetic nephropathy with adenosine levels and activity of ecto 5'-nucleotidase (CD73) that hydrolyses extracellular AMP. *Results:* After the third month of induction of experimental diabetes in rats, the renal injury is demonstrated by the presence of significant proteinuria and renal fibrosis markers. Urinary concentration of adenosine in rats progressively increased from the second month post-induction of diabetes. Also the same occurred in the activity of CD73 in urinary sediment. Immunohistochemistry showed an increased expression of CD73 at the renal tubular level as of the second month. CD73 enzyme activity in tubular extracts of kidneys also increased with the progression of diabetes in rats. *Conclusion:* The concentration of urinary adenosine and CD73 activity increase early on after the induction of experimental diabetes in rats, which precedes the evidence of renal damage such as proteinuria.

160) Evaluation of the overexpression of GDP dissociation inhibitor (*ScGDI1*) in *Arabidopsis thaliana* under salt stress.

San Martín, A[1]., Soto, F[1].,Pérez-Díaz, R[1].,Madrid-Espinoza, J[1].,González, E[1].,Ruiz-Lara, S[1].,[1]Instituto de Ciencias Biológicas Universidad de Talca. (Sponsored by FONDECYT 1140636)

Salinity is one of the most important factors which limiting crop productivity. With the aim of contributing to the knowledge of the molecular mechanisms involved in the salt tolerance we studied the *SchGDI1* from the salt tolerant wild tomato, *Solanum chilense* (Dunal) Reiche. *SchGDI1* showed a high induction when these plants were exposed to salinity stress. Phylogenetic analyses and multiple alignment showed a high homology with GDP- dissociation inhibitor (GDI) protein, which belong to RabGDI family. The Rab GTPases and GDI are key proteins involved in the intracellular vesicular trafficking. In order to evaluate the effect of heterologous expression of this gene, plants of *Arabidopsis thaliana* (Col-0) were transformed with 35SCaMV::SchGDI1. T1 lines were stressed under *in vitro conditions* with 100mM NaCl and evaluated in terms of some parameters such as: rate of germination, root elongation and degree of tolerance to salinity. Additionally, a bioinformatic approach was performed to determine the 3D structure for the GDI protein interacting with its putative target.

161) RIC-8B is important for neuronal differentiation of human Neuroblastoma SH-SY5Y cells

Sánchez, R[1]., Maureira , A[1].,Morin, V[1].,Torrejón , M[1].,Hinrichs, M[1].,Olate, J[1].,[1]Bioquímica y Biología Molecular, Ciencias Biológicas, Universidad De Concepción. (Sponsored by Facultad De Ciencias Biológicas, Universidad De Concepción)

Neuronal differentiation starts in early stages of embryonic development. In this process, neurons acquire a defined identity which is characterized by the position of neuronal body, the pattern of axonal projections and processes generation, a specific gene-set expression and metabolites synthesis. Although in neuronal differentiation many necessary proteins have been described, still uncharacterized proteins could play important roles. Here, we study the function of a Gα subunit regulatory GEF named RIC-8B, by overexpression and silencing assays using an *in vitro* differentiation model of human Neuroblastoma SH-SY5Y cell line. Cells were treated with all-trans retinoic acid and BDNF during a week, and the differentiation process was evaluated through morphological changes imaging, MAP-2 protein expression by western blot and NEU-N mRNA levels by RT-PCR. RIC-8B overexpression stimulates neuronal differentiation by acceleration the neurite outgrowth. Conversely, RIC-8B silencing inhibits SH-SY5Y differentiation progress to a neuro-like cell type. Theseresults show the importance of RIC-8B in SH-SY5Y cell differentiation and therefore we propose this protein asa potential candidate necessary for neuronal differentiation, and as an interesting target for neurodegenerative disease treatment.

162) A role for NHE1 in ovary cancer cell proliferation?

Sanhueza, C[1].,Araos, J[1].,Sáez, T[1].,Salsoso, M[1].,Pardo, F[1].,Leiva, A[1].,Cornejo, M[2].,Ramírez, M[2].,Sobrevia, L[1].,[1]Division of Obstetrics and Gynecology, Cellular and Molecular Physiology Laboratory (CMPL), Faculty of Medicine, Pontificia Universidad Católica de Chile.[2]Biomedical Deparment, Faculty of Health Science, Universidad de Antofagasta. (Sponsored by FONDECYT 3140516, 1110977, 11110059, 3130583.)

Ovary cancer results with abnormal cell growth of malignant cells. Cancer cells must adapt to low oxygen and acidity to proliferate. Prevention of intracellular acidification occurs mainly by H^+ extrusion where the Na^+/H^+ exchanger isoform 1 (NHE1) plays a crucial role enhancing cell proliferation. We evaluated NHE1 contribution to human ovary cancer cells proliferation in hypoxia. Ovary cell lines HOSE (normal), OVCAR3 and A2780 (tumor cells) and human primary cancer cells from ascitis (hCC) were exposed (0-48 h) to 20% O_2 (normoxia) or 1-10% O_2 (hypoxia). NHE1 mRNA expression, protein abundance and activity were assayed by qRT-PCR, Western blotting/indirect immunofluorescence and pHi recovery, respectively. Cell proliferation was measured in the absence or presence of zoniporide (NHE1 inhibitor) by hemocytometer cell counting. NHE1 was expressed in ovary tissue, and in normal and tumor cells. Cell proliferation was higher in 10% O_2 in tumor, but not normal cells compared with normoxia. Hypoxia increased NHE1 protein abundance, mRNA expression and NHE1 activity in A2780 cells. NHE1 inhibition by zoniporide reduced hypoxia-increased hCC (~50%) and A2780 (~25%) cell proliferation. Thus, ovary cancer cells exhibit a pro-proliferative response to hypoxia requiring NHE1 activity.

163) Role of the Sall2 tumor suppressor in the cell death response induced by TSA

Sanhueza, D[1]., Farkas, C[1].,Castro, A[1].,Pincheira, R[1].,[1]Laboratorio de Transducción de Señales y Cáncer, Departamento Bioquímica y Biología Molecular, Ciencias Biológicas, Universidad De Concepción. (Sponsored by FONDECYT 1110821)

Balance between acetylation and deacetylation of histones plays a critical role in the regulation of gene expression. Since altered expression of histone deacetylases (HDAC) have been linked to tumor development, HDAC inhibitors (HDACi) arose as additional promising therapeutic agents for multiple cancers. HDACi block the activity of HDACs, restore the expression of some tumor suppressor genes and induce growth arrest and apoptosis. However, the mechanisms by which these compounds work are still unclear. Studies from our laboratory indicate that the Sall2 transcription factor, a putative tumor suppressor plays a negative role in cell proliferation and induces apoptotic cell death under certain stress conditions. Here we investigate whether Sall2 plays a role in the cell death response induced by Trichostatin A (TSA), a well-known HDAC inhibitor, in breast cancer cells. Our data show that Sall2 promoter activity, mRNA and protein levels increase under TSA treatment. In addition, siRNA-mediated depletion of Sall2 decreased TSA-induced cell death in breast cancer cells, an effect that was correlated with diminished TSA-dependent upregulation of the pro-apoptotic gene NOXA1. Thus, our results suggest that Sall2 status is relevant for cancer treatments with HDACi.

164) Protein adsorption kinetics at single molecule level using atomic force microscopy imaging

Santander, A[1]., Naulin, P[1].,Barrera, N[1].,[1]Department of Physiology, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile.

Protein adsorption at solid surfaces is an important process in many research areas. In biotechnology, for example, understanding the process of adsorption will enable improved performance of new biomaterials and biosensors. Our experiments consist of proteins incubated into mica surface at different times and imaged via tapping-mode atomic force microscopy (AFM). These adsorbed molecules adopt the spherical cap shape with adsorption coefficient (height/diameter ratio) decaying exponentially at longer times. Using nanomechanical simulations of proteins adsorbed into flat surfaces it is shown a similar adsorption kinetics, which depends upon protein structure such as size and shape. Taken together these results represent the first experimental single molecule approach to characterize protein adsorption kinetics. Funded by Millennium Science Initiative P10-035F, Fondecyt 1120169 and Anillo ACT-1108 grants.

165) Myostatin receptor (ActRIB) modulates p85-PI3K activity during myoblast differentiation.

Saquel, C[1]., Fuentes, E[2].,Molina, A[2].,Valdés, J[1].,[1]Laboratorio de Bioquímica Celular, Facultad de Ciencias Biológicas. Interdisciplinary Center for Aquaculture Research (INCAR), Universidad Andrés Bello.[2]Laboratorio de Biotecnología Molecular, Facultad de Ciencias Biológicas. Interdisciplinary Center for Aquaculture Research (INCAR), Universidad Andrés Bello. (Sponsored by CONICYT/FONDAP/15110027)

Introduction: Myostatin, a member of the TGF-β superfamily, is a negative regulator of proliferation and differentiation of skeletal muscle cells.Myostatin exerts its inhibitory effect by binding to activin receptors inducing the phosphorylation of the transcriptions factors Smad2/Smad3 which ultimately leads to suppression of myogenesis. Here we provide evidence of a new mechanism involved in the inhibitory effect of myostatin in skeletal muscle growth through the direct modulation of IGF-1/PI3K/Akt signaling pathway. **Material and Methods:** Rat myoblasts were incubated with recombinant myostatin and/or IGF-1. Protein extracts were obtained and western blot realized to detect activation of downstream molecules of the IGF-1 signaling pathway. Immunoprecipitation assays were performed to detect interaction between ActRIB and p85-PI3K proteins. **Results:** Preincubation of myoblast with myostatin (2 nM) inhibits the phosphorylation of Akt and p85-PI3K induced by IGF-1 (10nM). Immunoprecipitation assays revealed that the interaction of ActRIB with p85 was induced by treatment with myostatin. **Discussion:** The present study support the hypothesis that myostatin inhibits myogenesis by a direct modulation of the IGF-1/PI3K/Akt signaling pathway, revealing unknown mechanisms of muscle growth in vertebrates.

166) Evaluation of the interaction of citrate with Fructose 1,6-bis phosphatase

Schott-Verdugo, S[1]., Asenjo, J[1].,Díaz, A[2].,Guinovart, J[3].,Slebe, J[1].,[1]Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral De Chile.[2]Instituto de Fisiología Celular, Facultad de Ciencias, Universidad Nacional Autónoma de México.[3]CIBERDEM Institute for Research in Biomedicine. (Sponsored by FONDECYT Grant 1090740;1141033)

FBPase is a key enzyme of the gluconeogenic pathway, since is one of the responsible of its regulation; it's inhibited by fructose 2,6-bisphosphate and AMP, which also activate phosphofructokinase (PFK). PFK is also activated by citrate, a metabolite with chelating properties. Studies of the 60' and 70' showed that FBPase is activated *in vitro* by chelating agents, responsibilizing the removal of trace amounts of Zn in the assay media for the observed activation. These studies were done with EDTA, and they didn't evaluate the effects of citrate in depth. Recently was showed that the *E. coli* enzyme is activated by citrate by interacting in the C_1-C_4 interface, stabilizing the tetramer, aptitude that theoretically has been lost in the eukaryotic enzyme because of changes in the composition of this interface. We evaluated *in silico* the interaction of citrate with pig kidney FBPase, finding a binding site equivalent to the *E. coli* enzyme. Mutants K42A and E192A, residues of the interface C_1-C_4, showed greater activation by citrate than the wild-type. FBPase crystalized with citrate showed a binding site in the C_1-C_4 interface, apparently displaced by glycerol. Assays with radioactive citrate showed binding with the E192A mutant, probably due to the removal of a negative charge of the interface. Even though the activation by remotion of inhibitor cations cannot be ruled out, the results show binding of citrate and suggest a role in FBPase activation.

167) Characterization of complexes that contain isochorismate synthase of *Arabidopsis thaliana*.

Seguel, A[1].,Jelenska, J[2].,Greenberg, J[2].,Holuigue, M[1].,[1]Genética Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica De Chile.[2]Department of Molecular Genetics and Cell Biology The University of Chicago. (Supported By FONDECYT (1141202) And Millennium Nucleus For Plant Functional Genomics (P10-062-F).)

The plant hormone salicylic acid (SA) regulates the plant defense system and accumulates in plant tissues subjected to a variety of biotic and abiotic stress conditions, such as pathogen infections and UV-C light exposure. The main source of SA under such conditions is the ICS pathway, a chloroplastic pathway in which Isochorismate synthase 1 (ICS1) is responsible for the conversion of chorismate to isochorismate. However, how the conversion from isochorismate to SA occurs in plants is still unknown. In some bacteria, it is known that ICS1 forms a complex in order to produce SA. Therefore, we hypothesized that ICS1 could work in a similar way in plants. To test this possibility, we performed immunoprecipitation of ICS1 and used mass spectrometry to identify co-purifying ICS1-interacting proteins (IIPs). In this analysis, we found a large number of peptides from a protein family that was previously described as mitochondria-localized. Here we report that IIPs also localize to chloroplasts, similar to ICS1. In a mutant lacking a major IIP isoform, the protein levels of a major SA-regulated gene, PR1, is reduced compared with WT plants and shows higher susceptibility to an avirulent pathogen. This evidence suggests that IIP could be involved in the SA-mediated response in *Arabidopsis thaliana*.

168) The activation process of the pain receptor TRPV1 is mediated by structural changes in S6-TRP domain

Sepulveda, R[2]., Latorre, R[1].,Gonzalez-Nilo, F[2,3].,[1]Centro Interdisciplinario de Neurociencia de Valparaíso Universidad De Valparaíso.[2]Center for Bioinformatics and Integrative Biology (CBIB), Facultad de Ciencias Biológicas, Universidad Andrés Bello.[3]Centro Interdisciplinario de Neurociencias de Valparaíso Universidad de Valparaíso. (This Work Is Supported By FONDECYT Grant 1131003 And CONICYT Doctoral Fellowships (To R.V.S.). The Centro Interdisciplinario De Neurociencia De Valparaíso Is A Millennium Institute Supported By The Millennium Scientific Initiative Of The Ministerio De Eco)

Transient receptor potential V1 (TRPV1) is a cation channel that recognize several kind of stimuli such as heat, pH, and endogenous vanilloids. The most known TRPV1 activator is a compound found in chili peppers: capsaicin, which interacts in a hydrophobic binding pocket located in the intersection of two subunits. Nonetheless, the role of another agonist of TRPV1, phosphatidylinositol 4,5-biphosphate (PIP_2), has been a source of several debates. The PIP_2 binding site has been studied using a strategy that combine mutagenesis assays and *in silico* analysis to identify the specific interactions between TRPV1 and PIP_2 and capsaicin. Moreover, TRPV1 activation response depends on the sequence at which these two molecules are added (Poblete et al, 2014).

In order to understand at molecular level that kind of interactions has been implemented molecular dynamics simulations of the TRPV1 structures in complex with capsaicin and/or PIP_2. Our results show a large conformational rearrangement in the S6-TRP domain, which would elicit the pore aperture and activation. This study provides new insights about the molecular mechanism involved in the TRPV1 activation.

169) Systematic identification of analogous enzymes in the diaminopimelate pathway for lysine biosynthesis

Sepúlveda, F[1]., Asenjo, F[1].,Álvarez, L[2].,Pérez-Donoso, J[2].,Almonacid, D[1].,[1]Center for Bioinformatics and Integrative Biology (CBIB), Facultad de Ciencias Biológicas, Universidad Andrés Bello.[2]Bionanotechnology and Microbiology Lab, Facultad de Ciencias Biológicas, Universidad Andrés Bello. (Sponsored by GRANT Regular UNAB DI-476-14/R)

There are two pathways for lysine biosynthesis; in plants, most bacteria and lower fungi, lysine is synthesized via the diaminopimelate pathway (DP), while in some bacteria (especially extreme thermophiles), yeasts and higher fungi, it is synthesized via the α-aminoadipate pathway. *C. glutamicum*, a microorganism used for industrial biosynthesis of amino acids, has the DP in which ten enzymes are responsible for the conversion of aspartate to lysine. For each gene in this pathway we performed blastp searches against the complete and reference proteomes in Uniprot. By doing this, we were able to obtain the homologous enzymes for each of the ten genes in each of those proteomes and identified organisms that contain all but one of the enzymes in the pathway. In these cases, the most parsimonious explanation is that the missing reaction is carried out by an analogous enzyme, an evolutionary scenario where the same enzyme reaction is performed by an enzyme from a different protein family to that in *C. glutamicum*. We have previously demonstrated that convergent evolution is very widespread, with two thirds of chemical reactions occurring in living organisms being catalyzed by enzymes from two or more different superfamilies. In this work, we evaluate the use of genomic context information from the Integrated Microbial Genomes, and of EC sub-subclass annotations from KEGG and Uniprot to identify analogous enzymes using the DP as example.

170) Interference of the antisense mitochondrial non-coding RNA decreases tumorigenic properties in human breast cancer cell lines.

Silva M, V[2,1,3]., Silva, V[2].,Socías, T[2].,Villegas, J[3,2].,Burzio, L[2,3].,Lobos-González, L[2].,[1]Escuela de Ingeniería en Biotecnología, Facultado de Ciencias Biológicas, Universidad Andrés Bello.[2]Andes Biotechnologies S.A. Fundación Ciencia y Vida.[3]Facultad de Ciencias Biológicas Universidad Andrés Bello. (Sponsored by Support: Andes Biotechnologies, Grant CCTE-PFB16 And Insertion Project 7812030019, CONICYT, Chile.)

Worldwide, breast cancer is the most common and deadly cancer in women. Available therapies are insufficient to eradicate this disease due to tumor heterogeneity, responsible for therapy resistance, disease progression and relapse. Our group described a novel family of human non-coding mitochondrial RNAs (ncmtRNAs) with Sense (SncmtRNA) and Antisense (ASncmtRNA) members. These RNAs are differentially expressed according to proliferative status of cells. Normal proliferating cells express both, but tumor cells down-regulate the ASncmtRNA. Knockdown of these transcripts with antisense oligonucleotides (ASO) induces selective and massive death of several human tumor cell lines of different origins. In this work, we knocked down the ASncmtRNA with a specific ASO in the human breast cancer cell lines MCF-7, ZR75-1 and MDA-MB-231, which represent cells with differential expression of molecular markers. Treatment for 48h induces 40-80% death, observed by trypan exclusion. Moreover, the invasive capacity is greatly reduced in matrigel invasion chambers. Finally, we observed that ASO treatment dramatically decreased mammosphere formation, indicating loss of anchorage-independent growth capacity. Therefore, knock-down of ASncmtRNAs decreases tumorigenic properties relevant to breast cancer development, constituting a preclinical approach for a future therapy against breast cancer.

171) Rab GTPases associated to tolerance to salt stress in tomato (*S. chilense*)

Soto, F[1]., San Martín, A[1].,Pérez-Diaz, R[1].,González, E[1].,Ruiz-Lara, S[1].,[1]Instituto de Ciencias Biológicas Universidad de Talca. (Sponsored by FONDECYT 1140636)

Rab GTPases are small GTPases that are key regulators of vesicle trafficking. Rabs function as molecular switches, cycling between GTP-bound and GDP-bound states. Cycling between GTP-bound/GDP-bound states is mediated by three major proteins classes: GAPs, GEFs and GDIs. Rab GTPases control docking, fusion and, in some cases, fission events during vesicle trafficking. To maintain proper traffic, each Rab member is considered to regulate a specific step in the complicated network of membrane traffic. In plants, Rab members consist of eight families (RabA-RabH). During salinity stress, plants need to re-establish homeostasis, protect and repair damaged proteins and membranes, which requires rapidly removal of existing molecules from various cellular compartments and replacing them with new ones. For this reason we search the Rab GTPases genes in the genome of S. lycopersicum based in those described for these genes in A. thaliana. These sequences were used to identify the Rab GTPase genes in wild tomato S. chilense, specie salinity tolerant. Additionally a phylogenetic tree was made to classify the Rab GTPases in the different sub-families. Also, 3D models were made and subjected to energy minimization and molecular dynamics to study the behavior and interaction with other proteins of the Rab cycle. In addition, expression analyses were performed to Rab GTPase genes of each sub-family in leaves and roots from S. chilense plants exposed to salt stress.

172) Angiontesin-(1-9) regulates mitochondria-endoplasmic reticulum communication in cardiomyocytes.

Sotomayor-Flores, C[1]., Rivera-Mejías, P[1].,Parra, V[2,3].,Pennanen, C[1].,Vasquez-Trincado, C[1].,Morales, P[1].,López-Crisosto, C[4].,Chiong, M[1].,Ocaranza, M[5].,Lavandero, S[1,2].,[1]Advanced Center for Chronic Diseases (ACCDIS), Facultad Ciencias Químicas y Farmacéuticas., Universidad de Chile.[2]UT Southwestern Medical Center, Medical sciences., University of Texas.[3]Laboratorio de transducción de señales moleculares, Facultad de Ciencias Químicas y Farmacéuticas., Universidad de Chile.[4]Advanced Center for Chronic Diseases (ACCDIS), Facultad de Ciencias Químicas y Farmacéuticas., Universidad de Chile.[5]Departamento Enfermedades Cardiovasculares, Facultad Medicina, Pontificia Universidad Católica de Chile. (Sponsored by FONDECYT 1120212 (SL), FONDECYT 3130749 (CP), FONDEF D11I1122 (MPO, SL, MC), FONDAP 15130011 (SL, MC) And CONICYT PhD Fellowship (to CFS, PR, CVT, PM CLC).)

Cardiomyocyte hypertrophy (CH) is an adaptive response to stress; however, chronic stress-induced hypertrophy leads to heart failure. CH is characterized by increases in sarcomere number and cardiomyocyte size, among changes in gene expression and energy metabolism. Our recent work showed that alterations in mitochondrial dynamics and Ca^{2+} homeostasis are associated with CH development. Angiotensin-1,9 (Ang-1,9) is a novel player of the renin-angiotensin system with antihypertrophic action. However it remains unknown how this peptide regulates CH. Our results showed that Ang-1,9, decreases and increases Ca^{2+} levels in cytosol and mitochondria, respectively, in cultured cardiomyocytes treated with histamine. Ang-1,9 also promotes mitochondrial fusion and prevents mitochondrial fission induced by the hypertrophic agonist Norepinephrine (NE). This effect was associated with a decrease in the fission protein Drp1 migration to mitochondria. Our data also revealed that Ang-1,9 prevents the loss of contact sites between mitochondria and ER and the increase in cytoplasmic Ca2+ levels induced by NE. We conclude that Ang-1,9 prevents alteration in Ca^{2+} homeostasis occurring in CH by controlling the communication between mitochondria and ER.

173) Preliminary biophysical characterization of the BiP chaperon protein, a protein involved in proteins translocation into the endoplasmic reticulum.

Tapia, A[1]., Vega, M[1].,Wilson C. A. M[1].,[1]Biochemistry lab, Biochemistry and Molecular Biology department, Faculty of Chemistry and Pharmaceutical Sciences, Universidad De Chile. (Sponsored by FONDECYT 11130263)

BiP is a chaperone located on the endoplasmic reticulum (ER) lumen and is involved in proteins translocation from the cytosol into ER in eukaryotic cells, which is the first step on protein trafficking; also, BiP takes part in the correct folding of these proteins. At present, the exact mechanism by which BiP participates in the translocation of polypeptides is unknown, since it is not clear if it uses the energy of ATP to generate mechanical motion pulling the polypeptide into the ER lumen or prevents that the incoming polypeptide to be returned to the cytosol. In our laboratory we have developed a strategy to purified BiP in only three steps by using Nickel and ATP-agarose columns. Then, we perform stability curves with guanidinium chloride (GdHCl) and follow the intrinsic fluorescence of tryptophan at different concentrations of GdHCl and found that BiP shows an increase in tryptophan fluorescence and a red shift of the maximum emission wavelength longitude as concentration of GdHCl have higher values. Thus, we have achieved a methodology to obtain pure BiP chaperon protein and also the results obtained with fluorescence showed that the tryptophan is buried in the protein structure and is a good probe to follow structural changes in this protein upon unfolding. Furthermore, by using other techniques as circular dichroism we expected to determine the number of intermediates that the protein has upon unfolding.

174) Phosphoinositide-localized biosynthesis links endocytic trafficking and heat shock response in *Arabidopsis thaliana*.

Tejos, R[1]., Rodriguez-Furlán, C[1].,Norambuena, L[1].,[1]Centro de Biologia Molecular Vegetal, Departamento de Biologia, Facultad de Ciencias, Universidad de Chile. (This Work Is Supported By The CONICYT Program Apoyo Al Retorno De Investigadores Desde El Extranjero 2012, PAI 82130047 (to RT))

Environmental stresses, such as temperature changes, drought or salinity, induce an array of cellular adjustments that facilitate plant acclimation and survival. Among the phosphorylated membrane lipids, the phosphatidylinositols (PtdIns) can be phosphorylated to generate potentially seven phosphorylated PtdIns forms, the so called phosphoinositides (PI). The dual PI function as scaffolding molecules and precursors of other secondary messengers, as well as their intracellular differential distribution, makes PIs important mediators of a wide variety of cellular processes like membrane trafficking, membrane homeostasis, nuclear signaling, and more prominently for stress responses. Here we show that a sudden increase in temperature (Heat Shock, HS) in *Arabidopsis thaliana* generates rapid changes in intracellular distribution of organelle and plasma membrane protein markers, and perturbs the uptake of the endocytic tracer FM4-64. We observe that the phosphoinositide markers YFP-$PH_{PLC\gamma1}$ [which labels PtdIns(4,5)P_2] and YFP-PH_{FAPP1} (a marker for PI4-P), together with the PI-metabolizing enzyme PIP5K1 are also modulated in content and subcellular distribution in response to HS. We discuss the data in the context of the role that PI relocalization and *de novo* metabolism may have on the subcellular response, the endocytosis modulation, and the overall plant heat shock response.

175) HERP regulates cardiomyocyte hypertrophy by controlling inositol triphosphate receptor

Torrealba, N[1]., Fernandez, C[1].,Paredes, F[1].,Pedrozo, Z[1].,Lavandero, S[2].,[1]Advanced Center for Chronic Diseases (ACCDiS) and Center for Molecular Studies of the Cell (CEMC), Faculty of Chemical and Pharmaceutical Sciences & Faculty of Medicine, Universidad de Chile.[2]Department of Internal Medicine, Southwestern Medical Center, University of Texas. (Supported By ACT 1111 (SL), FONDAP 15130011 (SL), FONDECYT 1120212 (SL), CONICYT PhD Fellowship 21120416 (NT).)

HERP is an endoplasmic reticulum (ER) membrane protein linked to ER-associated degradation. HERP has also shown to regulate Ca^{2+} homeostasis trough the degradation of the inositol triphosphate receptor (IP3R) during ER stress in neuronal models. Although HERP has been found in the heart, its function remains still not understood. Pathological cardiac hypertrophy can be triggered by a chronic activation of the sympathetic system via the activation of the α_1-adrenergic receptor/IP3R/Ca^{2+} signaling pathway. Moreover, IP3R is overexpressed during cardiac hypertrophy. Thus, we are investigating whether HERP prevents the development of cardiomyocyte hypertrophy by regulating IP3R protein levels.

To this end, cultured rat cardiomyocytes were treated with a siRNA against HERP in the absence or presence of the hypertrophic agonist norepinephrine (NE, 10 uM for 48 h). Our data showed that NE modulates HERP protein levels and its down-regulation by siRNA stimulates cardiomyocyte hypertrophy, increasing sarcomerization, cell perimeter and area as well as beta-myosin heavy chain levels in a similar manner to that observed with NE. Also, protein levels of IP3R increased after HERP knock-down, an effect that could be triggering the cardiomyocyte hypertrophic phenotype. These novel data support a new role for HERP in cardiac pathophysiology and establishes a new potential therapeutic target for the treatment of cardiac diseases.

176) Adenosine receptor 3 (ADORA3) control the survival and chemoresistance in glioblastoma multiforme

Torres, A[1,2]., Vargas, Y[1].,González, C[1].,Garrido, W[1].,Uribe, D[1].,Oyarzún, C[1].,San Martín, R[1].,Quezada, C[1].,[1]Instituto de Bioquímica y Microbiología, Ciencias, Universidad Austral De Chile.[2]Ciencias Veterinarias Universidad Austral De Chile.
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Glioblastoma multiforme (GBM) is the principal cause of death in brain cancer. Despite all efforts to improve the treatment, there is currently no cure, mainly due to the phenomenon of multiple drug resistance (MDR) through MRP1/MRP3 transporters, which makes ineffective conventional treatments. MDR has been associated with a subpopulation of Glioblastoma Stem-like Cells (GSCs) and high levels of adenosine. In order to evaluate this relationship, we use selective antagonists of adenosine receptors (ADORA) and CD73 (ecto-5'-nucleotidasethat produces adenosine), in combination with conventional anti-tumor drugs. These mixes were evaluated *in vitro* with adherent cells (Adh) and GSCs of human GBM (U87) and rat glioma (C6). Finally we evaluated the best combination *in vivo*. The antagonist of ADORA3 (MRS1220) and CD73 (AOPCP) were the only *in vitro* treatments that showed a decrease in cell viability. Moreover, MRS1220-Vincristine increased the percentage of apoptosis. GSCs showed increased resistance to treatment; however the combination of MRS1220 or AOPCP with anti-tumor drugs were able to reduce the cell viability. In both Adh and GSCs, MRS1220 and AOPCP decreased the expression and activity of MRP1/MRP3. *In vivo* tests showed that MRS1220 at 9 days of treatment decrease the tumor size (~90%), by decreasing the expression of proliferative markers and MRP1/MRP3. Based on these findings we propose the use of MRS1220 and AOPCP as a pro-apoptotic and chemosensitizing agents for GBM treatment.

177) Functional characterization of salicylic acid-inducible genes coding for glutathione *S*-transferases and glutaredoxins in the defense response to stress in *Arabidopsis thaliana*

Ugalde, M[1]., Fonseca, A[1].,Salinas, P[1].,Holuigue, M[1].,[1]Genética Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica De Chile. (Supported By FONDECYT (1141202) And Millennium Nucleus For Plant Functional Genomics (P10-062-F).)

Plants are organisms constantly exposed to several biotic and abiotic stress conditions that increase the production of reactive oxygen species (ROS) and alters the cellular redox state. The survival of the plants depend on a complex balance between the production and detoxification of ROS. Salicylic acid (SA) is a key phytohormone in the establishment of the defense response to stress, being essential for the production and also for the contention of the oxidative burst needed to establish the defense responses. SA induces the expression of genes coding for proteins with antioxidant and detoxifying function, among them *GLUTATHIONE S-TRANSFERASES* (*GSTs*) and *GLUTAREDOXINS* (*GRXs*). In this work, we performed an analysis of microarray databases to determine the expression pattern of *GSTs* and *GRXs* under different stress conditions where SA is involved. We identified 8 *GST* and 2 *GRX* genes and we confirmed their expression patterns induced by stress condition by real-time PCR. We selected mutant plants for *GSTU7*, *GRXC9* and *GRXS13* and evaluated their relevance to overcome different stress conditions such as treatments with methyl viologen, UV-C radiation and avirulent bacteria. Our results indicate that SA induces the expression of a set of *GST* and *GRX* genes in a temporal specific manner and that these genes are important in the contention of oxidative damage produced by different types of stress, suggesting a particular role for them in controlling ROS accumulation in the defense response.

178) Effect of hypoxia on multiple drug resistance in stem-like cells of glioblastoma

Uribe, D[1]., Torres, A[2,1].,Rocha, R[1].,Garrido, W[1].,Oyarzún, C[1].,San Martín, R[1].,Quezada, C[1].,[1]Instituto de Bioquímica y Microbiología, Ciencias, Universidad Austral De Chile.[2]Ciencias Veterinarias Universidad Austral De Chile. (Sponsored by FONDECYT N°1121121)

Glioblastoma multiforme (GBM) is considered the most aggressive and common brain tumor. One reason why chemotherapy fails is the Multiple Drug Resistance (MDR) phenomenon, caused mainly by the overexpression of ABC transporters (MRP1/MRP3). On the other hand, there is a subset of cells called glioblastoma like-stem cells (GSCs), which have been described as the primarily responsible of MDR in the hypoxic microenvironment. However, the mechanisms underlying the chemoresistance are not entirely clear. It has been reported that MDR could be mediated by adenosine signaling, since their bioavailability is increased in response to hypoxia. In this context, we postulate that hypoxic GSCs, overexpress Hypoxia-inducible factor (HIF) and thereby controlling MDR transporters via a mechanism mediated by adenosine. HPLC results show increased levels of adenosine and HIF-1α at 6, 24, 48 and 120 hrs of hypoxia compared to normoxia, which correlates with increased expression and activity of CD73-AMPasa. Through cytometry and MTT-assays, we found that MRS1220 (A3 receptor inhibitor), had the greatest effect on viability at 24 and 120 hrs of hypoxia (over 30% and 45%, respectively). MRP3 transporter expression increased 2-fold within 6 hrs of hypoxia, while expression of MRP1did not change. However, MRP3 overexpression is counteracted by using MRS1220. Together, these data suggest that the hypoxic microenvironment promotes MDR in GBM through a HIF1α-adenosine-mediated mechanism.

179) K_2P channels in plants and animals: similarities and differences

Valdebenito, B[1]., Caballero, J[1].,Janta, M[2].,Becker, D[2].,Riadi, G[1].,Gonzalez, W[1].,[1]Centro de Bioinformática y Simulación Molecular, Facultad de Ingeniería , Universidad De Talca.[2]Plant Molecular Biology & Biophysics University of Würzburg. (Sponsored by FONDECYT 1140624 And Programa CONICYT DAAD 2012)

Two-pore domain potassium (K_2P) channels are membrane proteins identified in mammals and other organisms such as plants. The functional channel is a dimer and each subunit has two pore-forming loops and four transmembrane domains. As K_2P channels in mammals (KCNKs), plant K_2P (TPKs) channels are target of external and internal stimuli to fine-tune the electrical properties of the membrane for specialized transport tasks. Although KCNKs and TPKs share the same topology, their sequence identity is low. Previous studies have led to the hypothesis that complex K^+ channels in plants and animals evolved from a simple bacterial precursor channel or from a viral-encoded K^+ protein. However, there are no current works performing a broad comparative analysis of K_2P channels. Here, we approach this topic by presenting phylogenetic, structural, and experimental evidence in order to understand the similarities and differences of K_2P channels. We hypothesize that K_2P channels probably originated after gene duplication in an ancient eukaryotic organism. Also, we show that KCNKs have structural features that are not present in TPK channels, such as a helical cap domain and lateral cavities exposing the ion-conducting pathway to the membrane. It may suggest that, although KCNK and TPK channels share the same topology and evolved from a common ancestor, their mechanisms of activation are different, indicating a specialization of potassium homeostasis in the animal and plant kingdoms.

180) Overexpression of the TcAP1 endonuclease ans its putative dominant negative in the viability of *Trypanosoma cruzi* Dm28c strain submitted to oxidative stress

Valenzuela, L[1]., Sepúlveda, S[1].,Ponce, I[1].,Bahamondes, P[1].,Galanti, N[1].,Cabrera, G[1].,[1]Biología Celular y Molecular, Facultad de Medicina, Universidad De Chile. (This Study Was Supported By Grant Fondecyt 1130113 (NG))

Trypanosoma cruzi, the causative agent of Chagas Disease, survives to DNA damage generated by ROS/RNS inside of their hosts. In recent eukaryotes, oxidative DNA damage is repaired mainly by Base Excision Repair (BER) pathway, being essential apurinic/apyrimidinic endonuclease activity of APE1. Nucleotide sequences that encode TcAP1 of *T. cruzi* (ortholog of human APE1) and a putative negative dominant of TcAP1 (TcAP1DN), obtained by site-directed mutagenesis, were amplified and inserted into the expression vector pTREX-GFP. Constructs were transfected in epimastigotes and then both endonucleases were submitted to *in vitro* metacyclogenesis to obtain amastigotes and trypomastigotes that express TcAP1-GFP and TcAP1DN-GFP. Protein expression was verified by direct microscopy, immunofluorescence and western blotassays. TcAP1 and TcAP1DN were purified in native conditions from transfected epimastigotes. AP endonuclease activity of TcAP1was determinated using a modified oligonucleotide labeled with P^{32}. Unlike APE1DN (human), TcAP1DN show a lower AP endonuclease activity and does not act as negative dominant. Parasites that overexpress TcAP1 increase its viability when are exposed to increasing concentrations of H_2O_2 for 30 minutes, while those parasites overexpressing a negative dominant do not show differences in its viability. Our results confirm that BER pathway is involved in the resistence of *T. cruzi* against oxidative DNA damage and suggests their participation in the persistence of the parasite in their hosts.

181) Chronic stress induces atrophy and immune response related gene expression changes in the skeletal muscle of the fine flounder under farming condition.

Valenzuela, C[1]., Zuloaga, R[1].,Fuentes, E[1].,Estrada, J[2].,Valdés, J[1].,Molina, A[1].,[1]Biotecnología Molecular, Ciencias Biológicas. INCAR (centro interdiciplinario para la investigación acuícola), Universidad Andrés Bello.[2]Centro de Investigación Marina Quintay (CIMARQ) Universidad Andrés Bello. (Sponsored by FONDECYT 1130545 And FONDAP INCAR 15110027.)

Introduction: In mammals acute stress inhibits muscle growth and promotes muscular atrophy. In addition, stress has a negative effect on the immune system generating a susceptibility to pathogens and diseases. However, little is known about the effects of chronic stress on muscle growth and which are the molecules and systems participating on this process.

Materials and Methods: We use as a model the fine flounder (*P. adspersus*), which were subjected to 4 and 7 weeks of confinement inducing chronic stress. Muscle and blood samples were collected from control and stressed fish at 4 and 7 weeks. The stress response was analyzed evaluating cortisol plasma levels by ELISA and corticosteroids receptors mRNA contents through qPCR (GR and MR). Markers of the immune response (TNFα, IL-1β, MHCI-II, TLR14 and TGFβ1), autophagy (Bnip3 and LC3 I-II), and the ubiquitin-proteasome system (Atrogin1 and Murf1) were also assessed.

Results: High cortisol levels were observed at 4 weeks of confinement. GR and Murf1 were up-regulated at 4 week. LC3 and Bnip3 were up-regulated at 7 week. Immune markers expression was negatively regulated. **Discussion:** The present results suggest that chronic stress affects muscle growth, down-regulating immune activity and promoting skeletal muscle atrophy via up-regulation of autophagy and ubiquitin proteasome system; however these mechanisms seems to have a temporal regulation according to the stress response.

182) Identification of functional promoters of two SL genes in *Cyprinus carpio*

Valenzuela, G[1]., Stolzenbach, Maria[1].,Figueroa, Jaime[1].,Alex, Romero[2].,Gudrun, Kausel[1].,[1]Instituto de Bioquímica y Microbiología, Ciencias, Universidad Austral de Chile.[2]Instituto de Patología Animal, Ciencias Veterinarias, Universidad Austral de Chile.

Somatolactin (SL), a fish specific pituitary hormone belonging to the prolactin (PRL) superfamily, is in volved in background adaptation, osmoregulation, reproduction and fatty acid metabolism. Two *sl* genes, α and β, were discovered in carp and transcripts of both were detected in pituitaries. Clearly, expression of *slα*and *slβ*was decreased significantly in pituitary of adult male carp in response to treatment with $ZnCl_2$, but only *slß* was induced upon 17β-estrogen (E2) treatment, relative to control carp as shown by RT-qPCR analyses. With the aim to characterize in detail the divergent regulation of the two SL genes, promoters of both genes were analyzed. Comparison of the complete coding sequences of *slα*and *slβ*revealed 61.6% identity at the nucleotide level and 46.2% between the derived aminoacid sequence. Promoter regions were obtained by a combination of in *silico* cloning and inverse PCR. To determine the proximal promoter región, transcription start site was detected with 5'RACE for 5´-UTR. In the proximal promoter putative binding sites for response elements to metal (MRE) and estrogen (ERE) were found with tfscan software. In addition, in both genes putative binding sites for Pit-1 were identified, suggesting regulation of SL by this pituitary specific transcription factor. Further studies will help to give insights in the transcription activator complex on the SL genes, which might reflect a situation of co-regulated factors in response to E2 or Zn in pituitary of carp.

183) Structural characterization and molecular dockingsimulation of FcEXPA1 and FcEXPA2 from *Fragaria chiloensis* (L). Duch.

Valenzuela-Riffo, F[1]., Gaete-Eastman, C[1].,Herrera, R[1].,Moya-León, M[1].,Morales-Quintana, L[1].,[1]Instituto de Ciencias Biológicas Universidad de Talca. (This Work Was Financed By Anillo ACT-1110. F.V. Acknowledges The Project Scholarship.)

Fruit softening has been shown to be related to cell wall degradation. As changes in the cellulose-hemicellulose fraction have been reported during ripening of *Fragaria chiloensis* fruit, the participation of expansins was studied. In previous studies two *expansins* genes were identified in *F. chiloensis* fruit with high homology to other plant α-expansins. Full-length sequences were named *FcEXPA1* and*FcEXPA2.*To gain insight about the mechanism of action of both proteins at the molecular level, the comparative modeling methodology was employed to build the enzymes structures, which were validated and refined with molecular dynamics simulation (MDS). The models displayed similar structures comprising 13 β-sheets and 2 α-helices for FcEXPA1 and 16 β-sheets and 2 α-helices for FcEXPA2. Additionally, the catalytic motif **HFD** is oriented towards the central of the open groove in the two structures. The interaction of a set of putative xyloglucan substrates (XG) and a cellulose octamer with the proteins was explored using molecular docking and MDS with MM-GBSA. Both enzymes showed favorable affinity energies for binding XG and cellulose substrates, however the best stability complex was with the XXFG substrate (-73.7 kcal/mol and -79.1 kcal/mol in FcEXPA1 and FcEXPA2, respectively). The higher energy contributors in the final binding energy are the Van der Waals and non-polar terms. The data is congruent with a probable role of expansins during strawberry fruit development and ripening.

184) Autophagy flux is impaired in fibers isolated from a Duchenne muscle model.

Valladares, D[1]., Utrera-Mendoza, Y[2].,Wesrermeier, F[1].,Jaimovich, E[2].,Lavandero, S[1].,[1]Bioquímica y Biología Molecular, Ciencias Químicas, Universidad De Chile.[2]Biología Celular, Medicina, Universidad De Chile. (Sponsored by FONDECYT 3140491 (DV), 3140532 (FW), 11467 (EJ), ACT-111 (EJ, SL), FONDAP 15130011 (SL))

There is an increasing interest in the study of autophagy for the treatment of skeletal muscle diseases, including Duchenne muscular dystrophy (DMD). Nevertheless until now there is no detailed study in autophagy flux in DMD. Our aim was to investigate basal autophagy levels and autophagy flux in fibers from a DMD animal model, *mdx*. For this end we analyzed mRNA and protein levels of LC3, p62, Beclin and Bcl-2 in FDB muscle of mdx (4-6 weeks). Autophagy flux was evaluated by the formation of LC3 and p62 puncta using different stimuly like starvation and rapamycin in the presence or absence of bafilomycin. At basal levels the expression of LC3II are diminished in mdx fibers compared with controls. This result correlates with an increase expression of p62 in mdx fibers. We also found differences in other autophagy proteins like Beclin1, Bcl-2 and Bnip3 among others. By immunofluorescence we observed differences in the basal LC3 and p62 puncta similar to the differences observed in the expression of these proteins. The fibers under starvation in the presence of bafilomycin responded by increasing LC3 and p62 puncta both in control and mdx fibers. However the mean of change was greater in the control than in mdx fibers. Similar results were obtained with rapamycin. Collectively, these results suggest that autophagy flux is altered in mdx fibers and could be an important mechanism for the muscle weakness observed in DMD patients.

185) Stability of the cold shock protein from *Bacillus caldolyticus*: role of the hinge section.

Vallejos, G[1]., Ramirez-Sarmiento, C[1].,Babul, J[1].,[1]Departamento de Biología, Facultad de Ciencias, Universidad de Chile.

The cold shock protein from *Bacillus caldolyticus* (*Bc*CSP) is a small single-domain protein whose structure corresponds to a compact five-stranded β-barrel organized in two subdomains facing each other to form the protein's hydrophobic core.

Recently, a crystallographic structure of *Bc*CSP was obtained as a domain swapped (DS) dimer. The exchanged segment corresponds to an entire subdomain and the hinge region consists of only two residues (E36 and G37). Experimental and structural evidence in *Bc*CSP and homolog proteins suggest that, in addition to determining the DS dimer formation, hinge residues are also important for protein stability.

To address these phenomena, we performed structure-based simulations and site-directed mutations of hinge residues E36 and G37 to proline in *Bc*CSP. Our simulations show that monomer protein topology determines folding into the DS dimer seen in the crystal structure. However, size-exclusion chromatography shows that both wild type *BcCSP* and hinge mutants are monomers in all tested conditions. To determine the effects of the hinge section in protein stability, we carried out thermodynamic studies through equilibrium unfolding and refolding. A destabilization of 1 kcal·mol^{-1} for G37P and an increase in 0.34 kcal·mol^{-1}·M^{-1} of the m-value of E36P-G37P was determined. Our results suggest that the hinge section plays a role in stability, but might not be fundamental for folding of BcCSP as a monomer or DS dimer. Fondecyt 1130510.

186) DYRK1B expression in Sertoli cells: potential mechanism involved in the regulation of glycogen synthesis.

Vander Stelt, K[1]., Covarrubias, A[1].,Mancilla, H[1].,López, C[1].,Cereceda, K[1].,Angulo, M[1].,Slebe, J[1].,Concha, I[1].,[1]Instituto de Bioquímica y Microbiología, Ciencias, Universidad Austral De Chile. (Sponsored by FONDECYT 1110508 (IC), 1141033 (JCS), Beca CONICYT KV.)

Sertoli cells provide structural and nutritional support for the developing germ cells and are able to synthesize glycogen, main energy reserve for cells. Glycogen synthesis is a highly regulated process in which Muscular Glycogen Synthase (MGS) is phosphorylated on nine or more sites by multiple protein kinases and leads to the inactivation of MGS. The critical phosphorylation site that controls 80% of enzyme activity is Ser640 (site 3a) which is directly phosphorylated by a Dual-specificity tyrosine phosphorylation-regulated kinase (DYRK) family. DYRK1B is a specific isoform expressed in the seminiferous epithelium. The aim of this work was to characterize DYRK1B in pre puber and adult Sertoli cells and analyze the potential role in the inhibition of glycogenesis. By RT-PCR and Western blot analysis we determined the expression of DYRK1B in these cells and by qRT-PCR we observed no differences at the transcript level. Confocal microscopy showed that DYRK1B localized in the nucleus and cytoplasm, this was confirmed by subcellular fractionation of nuclei and cytosol. Additionally, MGS was localized in both compartments and was in a highly phosphorylated state. Immunofluorescence assays revealed perinuclear colocalization of DYRK1B and MGS. Finally, we used AZ191, which selectively inhibits DYRK1B in in vitro assays, suggesting that DYRK1B phosphorylates MGS at Ser640. These results indicate for the first time that in testis, glycogen synthesis is being regulated by a recently described kinase.

187) Tellurite reductase activity of *Escherichia coli* flavoproteins and biosynthesis of tellurium-containing nanostructures

Vargas-Pérez, J[2]., Arenas, M[1].,Muñoz, C[2].,Cornejo, F[2].,Díaz-Vásquez, W[2].,Valdivia, M[2].,Vásquez, C[2].,Arenas, F[2].,[1]Centro de Bioinformática y Simulación Molecular, Ingenieria, Universidad De Talca.[2]Biologia, Quimica y biologia, Universidad De Santiago De Chile. (Financial Support: FONDECYT Postdoctorado 3120049 And FONDECYT Regular 1130362.)

Tellurite is toxic to most organisms at very low concentrations (nM). However, it has been observed that a bacterial resistance mechanism to this toxicant is its enzymatic reduction to elemental, non-toxic form in a reaction coupled to NAD(P)H oxidation (Tellurite Reductase activity or TR). To date, a number of enzymes exhibiting TR activity such as catalase, terminal oxidases, nitrate reductases and dihydrolipoamide dehydrogenase (LpdA) have been described. A bioinformatic analysis of these proteins allowed to identify six *E. coli* flavoproteins that putatively exhibit TR activity: thioredoxin reductase (TrxB), alkyl hydroperoxide reductase (AhpF), putative oxidoreductase (YkgC), glutathione reductase (GorA), nitrite reductase (NirB) and flavorubredoxine reductase (NorW). Their genes were cloned, overexpressed and the proteins purified and characterized. All of them exhibit TR activity *in vitro* at the expense of NADH and/or NADPH oxidation. Their optimal pH and temperature is 8-9 and 42°C, respectively. GoR's and AhpF's TR activity generated nanostructures *in vitro* with an average diameter of 75 nm, while LpdA and YkgC generated larger structures (over 100 nm). It was also possible to generate nanostructures *in vivo* by using strains that over express genes encoding TrxB, GorA and YkgC. These appeared as electron dense deposits in cells previously exposed to tellurite.

188) Extracts of *Thuja occidentalis* and fractions α/β-thujone as putative chemosensitizer in brain cancer

Vargas, Y[1]., Torres, A[1,2].,Erices, J[1].,Quezada, C[1].,[1]Instituto de Bioquímica y Microbiología, Ciencias, Universidad Austral De Chile.[2]Ciencias Veterinarias Universidad Austral De Chile. (Sponsored by Fondecyt N°1121121.)

Thuja occidentalis (*T. occidentalis*)is used in homeopathy for the treatment of resistant tumors by an entirely unknown mechanism of action. At present, there is a lack of studies to evaluate the effect of *T. occidentalis* in glioblastoma multiforme (GBM), the worst prognosis brain cancer. We have reported the expression of proteins of the ABC transporter superfamily that confer the multidrug resistant (MDR) phenotype in GBM. Based on this, we set out to study the antineoplastic effect of *T. occidentalis* and itsfractions (α and α/β-thujone) in human GBM (U87), rat glioma (C6) cells and *in vivo* model. Using HPLC, we found that *T. occidentalis* is rich in terpenes (α/β-thujone), showing a marked decrease in cell viability and cellular proliferation, without affecting control cells (SVG p12). α-thujone had no effect in cell viability, but α/β-thujone showed a potent effect in cell viability, apoptosis and proliferation. Moreover, α/β-thujone fraction exhibited a potent anti-angiogenic effect, and decreased the expression and activity of the MRP1 transporter. Finally, *in vivo* assays showed that α/β-thujone fraction only decreased tumor volume. In conclusion, *T. occidentalis* extract is rich in terpenes, showing a similar antineoplasic effect in human GBM and rat glioma cells way, without affecting control cells. We suggest the use of this extract or α/β-thujone fraction as a pro-apoptotic, anti-angiogenic and putative chemosensitizer in brain tumors.

189) Sortin2 enhances endocytic trafficking towards the vacuole in *Saccharomyces cerevisiae*

Vásquez-Soto, B[1]., Cruz-Amaya, M[1].,Manriquez, N[1].,Rubilar-Hernández, C[1].,Zouhar, J[2].,Raikhel, N[3].,Norambuena, L[1].,-[1]Centro Biología Molecular Vegetal, Facultad de Ciencias, Universidad De Chile.[2]Centro de Biotecnología y Genómica de Plantas Universidad Politécnica de Madrid, Madrid, España.[3]Center for Plant Cell Biology and Department of Botany and Plant Sciences University of California.Riverside, CA 92521. USA. (Sponsored by FONDECYT 11080240 And 1120289 (to BV, MC, NM, CR And LN), PCB-MN P006-065-F (to NM And LN) And NSF MCB0515963 (to JZ And NVR). Beca CONICYT Doctorado Nacional. Beca CONICYT Asistencia A Congreso.)

A highly regulated trafficking of cargo vesicles in eukaryotes performs protein delivery to a variety of cellular compartments. Developing new tools to modulate protein trafficking allows better understanding the endomembrane system regulation. The compound Sortin2 has been described as a protein trafficking modulator affecting targeting of the vacuolar protein carboxypeptidase Y (CPY), triggering its secretion in *Saccharomyces cerevisiae*. In this study, a reverse chemical-genetics approach was used to identify key proteins for Sortin2 bioactivity. A genome-wide Sortin2 resistance screen revealed six yeast deletion mutants that do not secrete CPY when grown at Sortin2 condition where the parental strain does: *met18*, *sla1*, *clc1*, *dfg10*, *dpl1* and *yjl175w*. Integrating mutant phenotype and gene ontology (GO) annotation of the corresponding genes and their interactome pointed towards a high representation of genes involved in endocytosis. In wild type yeast endocytosis towards the vacuole was faster in presence of Sortin2, which further validates the data of the genome-wide screen. This effect of Sortin2 depends on its structural features, suggesting compound specificity. Sortin2 did not affect endocytic trafficking in Sortin2-resistant mutants, suggesting that the Sortin2 effects on the secretory and endocytic pathways are linked.

190) A review of the structure of R-Phycocyanin from *Gracilaria chilensis*

Vásquez, J[1,2]., Lobos , F[1].,Bunster, M[1].,Martínez-Oyanedel, J[1].,[1]Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad De Concepción.[2]Programa de Doctorado en Ciencias Biologicas, Ciencias Biologicas, Universidad de Concepcion. (Sponsored by Fondecyt 113.0256)

In order to elucidate the actual sequence of phycocyanin (PC) present in *Gracilaria chilensis* and improve the understanding of their participation in the phycobilisomes as light sensors systems, it has been proposed to determine the sequence of their genes to compare primary with those obtained from the crystal structure deposited in the Protein Data Bank (PDB) under the code 2bv8. Mass spectrometry (MS) was used to obtain partial primary structure of Phycocyanin to design primer to amplify CPCA and CPCB genes encoding the α and β subunits of PC. After sequencing PCR products, with sizes of 486 (CPCA) and 516 bp (CPCB) we found 15 and 10 changes in the primary structures of a and b subunits, respectively. These changes were reviewed in the electron density map of the crystal structure and all of them agree in the map. Further refinement of the model and a short molecular dynamics protocol shown that these changes do not disturb the heterodimer or hexamer structure of Phycocyanin.

191) Lipotoxic stress increases mitochondrial E3 ligase Mul1 in cultured cardiomyocytes

Vásquez-Trincado, C[2,3]., Campos, C[1].,Rivera-Mejías, P[2,3].,Westermeier, F[2,3].,Navarro-Márquez , M[2,3].,Espinosa, A[1].,Lavandero , S[2,3].,[1]Escuela de Tecnología Médica, Facultad de Medicina, Universidad de Chile.[2]Advanced Center for Chronic Diseases (ACCDiS), Facultad de Ciencias Químicas y Farmacéuticas - Facultad de Medicina, Universidad de Chile.[3]Center for Molecular Studies of the Cell (CEMC), Facultad de Ciencias Químicas y Farmacéuticas - Facultad de Medicina, Universidad de Chile. (Sponsored by ACT 1111 (SL), FONDAP 15130011 (SL), FONDECYT 1120212 (SL), CONICYT PhD Fellowship (C-VT, P-RV, M-NM).)

The metabolic disorders are a major cause of cardiovascular disease. Saturated fatty acids are among the factors causing severe perturbations at the heart. This lipotoxic stress generates metabolic and mitochondrial disturbances and also insulin resistance. In this pathological state, FoxO transcription factors are principally active, producing significant changes in cardiac morphology, function and metabolism. One of FoxO downstream genes is the E3 mitochondrial ligase Mul1, which produces mitochondrial fission and negatively regulates Akt kinase, a key component in insulin signaling. To study a possible link between the effects of lipotoxic stress and Mul1 in the heart, we set up an *in vitro* model of lipotoxic stress, culturing cardiomyocytes with the saturated fatty acid myristic acid (MA). Cardiomyocytes stimulated with MA manifested cardiac hypertrophy and insulin desensitization with a decreased Akt activation, recapitulating the effects of a high fat diet on the heart. Furthermore, cardiomyocytes presented a fragmented mitochondrial network as lower levels of mitochondrial fusion protein Mfn2. In this *in vitro* context, MA increased Mul1 mRNA and protein levels. Collectively, these results suggest a possible involvement of Mul1 in the effects of lipotoxic stress in the cardiac tissue.

192) Mineralocorticoid hypertension markers are associated with immune activation molecules.

Vecchiola, A[5,4].,Cifuentes, M[1].,Lagos, C[4].,Fuentes, C[4].,Campino, Carmen[4].,Allende, Fidel[2].,Solari, Sandra[2].,Carvajal, Cristian[4].,Kalergis, Alexis[3].,Fardella, Carlos[4,5].,[1]Nutrition & Food Technology Institute (INTA) Universidad De Chile.[2]Laboratorios Clínicos, Escuela de Medicina, Pontificia Universidad Católica De Chile.[3]Molecular Genetics & Microbiology, Ciencias Biológicas, Pontificia Universidad Católica De Chile.[4]Endocrinología, Escuela de Medicina, Pontificia Universidad Católica De Chile.[5]Millennium Institute of Immunology and Immunotherapy Pontificia Universidad Católica De Chile.
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Recent evidence supports a role of inflammation and immunity in the development of hypertension. Aldosterone can directly alter the function of the immune system and cause vascular damage. **Goal:** To evaluate if mineralocorticoid hypertension markers, plasma renin activity (PRA) and aldosterone, as well as renal function markers as Naur, FENa%24h and blood pressure are related to immune signaling molecules Hsp70, Hsp90, TLR-2, TLR-4 and CD-14 expression in human circulating monocytes.**Methods:** 200 individuals (9-67 years old, BMI 26.8 ± 5.1 kg/$m^{2,}$ 61% female) blood pressure were registered. PRA (ng/mL*h), aldosterone (ng/mL), were measured on blood samples. urNa (mEq/creatinine mg) and FENa % at 24h where measured in urine samples. RNA from peripheral blood mononuclear cells (PBMCs) was isolated and expression of Hsp70, Hsp90, TLR2, TLR4 & CD14 was evaluated by q-RT-PCR. Aldosterone to PRAratio (ARR) was calculated. These data were analyzed by either Spearman or Pearson correlation, $p<0.05$ was considered significant.**Results:** PRA was associated with Hsp70, Hsp90, TLR4 and CD14 expression and showed a tendency with TLR2. Aldosterone was associated inversely to CD14. ARR was associated inversely to TLR4. urNa was associated with Hsp90 and TLR2. FENa was inversely related to TLR4. Finally PAD was inversely associated with CD14 and it showed a tendency with PAS. **Conclusion:** PRA, Aldosterone and ARRassociate with Hsp70, Hsp90, TLR-4 and CD-14 mRNA expression These immune activation molecules could be a early biomarker of an hypertensive condition.

193) Effects of insulin and D-glucose on theactivity of equilibrative nucleoside transporter in ratGLOMERULI

Vega, G[1]., Alarcón, S[1].,Quezada, C[1].,San Martín, R[1].,[1]Instituto de Bioquímica y Microbiología, Ciencias, Universidad Austral de Chile. (Supported By FONDECYT 1130414, CONICYT 21100738)

Introduction. Diabetic nephropathy (DN) is one of the most devastating kidney diseases, and remains incurable to date. Elevated levels of extracellular adenosine have been associated with interstitial progression of fibrosis in chronic kidney disease animal models. It has been reported that one of the major mechanisms by which the extracellular availability of adenosine is regulated involves the expression and activity of the equilibrative nucleoside transporters (ENTs). Our aim was to evaluate the functional effect of D-glucose and insulin on ENTs. *Results.* Our results show that high D-glucose concentrations decrease both ENT1 and ENT2 activities in glomeruli, podocytes and mesangial cells. Furthermore, insulin was able to reverse this effect by restoring the activity of these transporters to baseline levels. However, we could not detected changes in ENT1 or ENT2 expressions in total protein extracts from the different cell types studied when treated with insulin or D-glucose. Conversely, we noted that these stimuli differentially regulate plasma membrane localization of these proteins. *Conclusions*. Together, these data suggest that changes in adenosine levels are regulated by differences in subcellular localization and activity of ENT1 and ENT2, triggered by high D-glucose concentration and deficient insulin activity in diabetes.

194) Transcription-independent patterns in histone H3 heritable marking

Veloso, F[1]., Montecino, M[1].,[1]Center for Biomedical Research, Faculty of Biological Sciences, Universidad Andrés Bello, FONDAP Center for Genome Regulation.

There is wide scientific consensus on the physiological relevance of epigenetic information and epigenetic control mechanisms. This consensus is mainly based on the well-established association between the presence of particular histone post-translational profiles and gene transcription activity. Nevertheless, attempts to explain the presence of particular epigenetic states in a given locus respect to other epigenetic profiles present at other loci raise the question of how those epigenetic states can be ultimately and coordinately, regulated. Here, we have performed extensive bio-informatics analyses on public databases seeking for new constraints associated with the presence and distribution of specific histone marking throughout the genome, but that also are independent of gene transcription activity. We report the existence of additional information profiles associated with human, mouse, fruit fly and nematode histone H3 epigenetic modifications that are both heritable and explicitly uncorrelated to transcriptional states. We have defined these transcriptionally-independent patterns of histone modifications as 'paragenetic' information. Under a general theory, we propose that paragenetic information represents in these four metazoans a key feature of their differentiated multicellularity. Specifically, paragenetic information may be in tight correlation with the dynamics of the embryonic developmental process from totipotent stem cells, as well as with the emergence of differentiated multicellular phyla during evolution.

195) Calreticulin silencing decreases the viability of human ovarian cells

Vera, C[1]., Poblete, C[1].,Vega, M[3,1].,Ferreira, L[2].,Romero, C[3,1,4].,[1]Laboratorio de Endocrinología y Biología de la Reproducción, Hospital Clínico Universidad de Chile, Universidad De Chile.[2]Programa de Inmunología, ICBM, Facultad de Medicina, Universidad De Chile.[3]Departamento de Ginecología y Obstetricia, Facultad de Medicina, Universidad De Chile.[4]Advanced Center for Chronic Diseases (ACCDiS) . (Sponsored by FONDECYT 1110372 (C.R.) CV: Becaria Doctorado Nacional)

Ovarian cancer is the fifth most common cancer in women; it's characterized by a poor prognosis and low response to therapy. Calreticulin (CRT) is a multifunctional chaperone of the endoplasmic reticulum. It can also be found in other cellular compartments, including the cytosol, nucleus, secretory granules, the cell membrane and the extracellular matrix. In different types of cancer, calreticulin levels have been associated with both good and poor prognosis. In human ovarian cancer, calreticulin levels are elevated. In order to determine whether calreticulin provides to ovarian cells survival advantages or disadvantages, we transiently silenced calreticulin expression and then measured cell viability. We silenced two cell lines, human ovarian epithelial cells (HOSE) and ovarian cancer cells (A2780), using two siRNA sequences. After 24, 48 and 72 h, silencing efficiency was determined by western-blot and cell viability was measured using compound ab112118. We found a viability reduction in A2780 cells after CRT silencing, especially at shorter times. Viability was also reduced in HOSE cells, and the effect seems to be more durable, however, it is also less evident. CRT effects in cancer are still not clear, which could be attributed to its many function. This preliminary results indicate that the increase on calreticulin levels found in ovarian cancer tissues give advantages to the tumor cells, and that CRT expression is more important in malignant than in normal cells.

196) Gene regulatory networks underlying developmental adaptations in response to changes in nutrient availability in *Arabidopsis thaliana*

Vidal, A[1]., Moyano, T[1].,Montecinos, A[1].,Kraiser, T[1].,Gutiérrez, R[1].,[1]Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile. (Sponsored by FONDECYT 11121225 And 1141097, International Early Career Scientist Program From Howard Hughes Medical Institute, FONDAP Center For Genome Regulation 15090007, Millennium Nucleus Center For Plant Functional Genomics P10-062-F)

As sessile organisms, plants have developed sophisticated mechanisms to adapt to environmental conditions which include continuous sensing of their surrounding environment and transduction of these signals into modulation of growth, development and physiology. One of the most important environmental conditions that control organismal responses is nutrient availability. Although there have been significant advances into dissecting how nutrient signals are sensed and transduced by plants, these studies analyze the effect of nutritional changes in a specific time point of the plant life. Plants produce different types of organs at different times throughout their life cycle, thus these different stages of development should have different nutritional demands. Since each of these developmental stages is expected to express specific genes and gene regulatory networks (GRNs), it is expected that plant responses to nutrients vary accordingly over their life cycle. In this work, we aim to identify GRNs underlying plant development and phase change and how these GRNs are affected by changes in nutritional conditions using *Arabidopsis thaliana* as a model system. We will take changes in nitrogen (N) availability as an example of nutritional perturbation since N is an essential macronutrient and a key regulator of plant growth and development. Our preliminary data suggests that changes in N concentration might control Arabidopsis development by regulating key nitrate responsive genes involved in developmental transitions.

197) The southern amerindian genome reveals links to prevalent diseases in native and admixed latin americans.

Vidal, A[1]., Moyano, T[1].,Moraga, C[1].,Bustos, B[2].,Pérez-Palma, E[2].,Montecinos, A[1].,Azócar, L[3].,Vidal, M[1].,Di Genova, A[4].,-Buch, S[5].,Hampe, J[5].,Allende, M[6].,Cambiazo, V[7].,González, M[7].,Hodar, C[7].,Montecino, M[2].,Muñoz, C[8].,Orellana, A[8].,Reyes-Jara, A[7].,Travisany, D[4].,Veloso, F[2].,Vizoso, P[8].,Moraga, M[9].,Eyheramendy, S[10].,Maass, A[4].,De Ferrari, G[2].,Miquel, J[3].,-Gutiérrez, R[1].,[1]Departamento de Genética Molecular y Microbiología Pontificia Universidad Católica De Chile.[2]Centro de Investigaciones Biomédicas Universidad Andrés Bello.[3]Departamento de Gastroenterología Pontificia Universidad Católica De Chile.[4]Departamento de Ingeniería Matemática y Centro de Modelamiento Matemático Universidad De Chile.[5]Medical Department I University Hospital Dresden.[6]Departamento de Biología Universidad De Chile.[7]Laboratorio de Bioinformática y Expresión Génica INTA.[8]Centro de Biotecnología Vegetal Universidad Andrés Bello.[9]Instituto de Ciencias Biomédicas y Departamento de Antropología Universidad de Chile.[10]Departamento de Estadística Pontificia Universidad Católica De Chile. (Sponsored by FONDAP Center For Genome Regulation 1509000, FONDECYT 1130303, 1100942, 1120813)

Native American and Mestizo populations with Amerindian ancestry are more susceptible than other peoples to develop diseases related to metabolic disorders. This increased susceptibility could be explained in part by genetic variants transmitted from Native Americans to admixed populations. However, there is scant information on the genomic structure of Native Americans, especially those from the Southern Cone. Herein, we obtained a high quality complete genome sequence of 11 Mapuche-Huilliche individuals from Southern Chile and showed that this cohort represents an original non-admixed American population. We identified a considerable number of new genetic variants,some of which are predicted to alter gene function. Our analysis uncovered variations in functionally linked genes that might lead to complex metabolic diseases. Accumulation of mutations in specific pathways might explain the increased risk of common diseases in both Native American and Latin American populations.

198) Host cell transcriptome during Bovine Viral Diarrhea Virus Type 1 acute infeccion reveals new aspects of early genes expression.

Villalba, M[1]., Fredericksen, F[1].,Silva, A[2].,Yañez, A[1].,Olavarria, V[1].,[1]Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile.[2]AustralOmics. Instituto de Bioquímica y Microbiología Universidad Austral de Chile. (Sponsored by INNOVA-CORFO 12IDL2-16212.CONICYT-PCHA/Doctorado Nacional/2014)

Bovine viral diarrhea virus (BVDV) belongs to the genus Pestivirus of Flaviviridae family and is the causative agent of a group of complex diseases including mucosal disease, reproductive failure and birth defects in calves, persistent bovine infections and immunosuppression. BVDV initially enters epithelial cells, lymphocytes and monocytes, where it replicates and spreads in the lymphatic system, impairing the immunity of the infected animal. BVDV can affect almost every organ system in the body. Then, is important to know the characteristics of the virus-host of the complex etiology of the disease interactions. The availability of the host sequences makes them suitable targets for analysis tools to assess the whole genome of these interactions. Therefore, we propose to determine whether BVDV-1 acute infection can alter the expression of host early genes. To assess these, we performed a meta-transcriptomic analysis generated by pyrosequencing and in silico analysis where evaluated at transcriptional level, changes in early expression of cellular components against BVDV-1 infection. BVDV infected cells marked up-regulation of numerous genes belonging to diverse functional classes, including endoplasmic reticulum stress-inducible genes, actin cytoskeleton signalling, clathrin mediated endocytosis signaling, and showed weak induction of IFN-stimulated genes as we expected. Surprisingly, we found up-regulation of Wnt signaling involved in embryonic development and overexpression of genes involved in ovarian differentiation.

199) Insulin resistance in newborn vascular tissue form maternal obesity pregnancies

Villalobos-Labra, R[3]., Westermeier, F[1,3,2].,Sáez, P[3].,Salsoso, R[3].,Kusanovic, J[3].,Poblete, J[3].,Mardones, F[4].,Sobrevia, L[3,5].,-Farias, M[3].,[1]Escuela de Química y Farmacia, Facultad de Ciencia, Universidad San Sebastián.[2]Advanced Center for Chronic Diseases (ACCDiS), Faculty of Chemical & Pharmaceutical Sciences & Faculty of Medicine, Universidad de Chile.[3]Obstetrics and Gynaecology, Faculty of Medicine, Pontificia Universidad Católica De Chile.[4]Division of Public Health, Faculty of Medicine, Pontificia Universidad Católica De Chile.[5]University of Queensland Centre for Clinical Research (UQCCR), Faculty of Medicine and Biomedical Sciences, University of Queensland. (Sponsored by FONDECYT (1 121145,1110977,1090594,3140532), CONICYT(ACT-73 PIA))

Maternal obesity (MO) has been associated with development of obesity and diabetes mellitus in the offspring. Because insulin resistance (IR) is a key mechanism of those adverse outcomes, here we evaluated whether MO is related to early changes in the insulin response of neonatal tissues. Primary cultures of human umbilical vein endothelial cells (HUVEC) were isolated from normal (HUVEC-N) or MO (HUVEC-OB) pregnancies, attending to obstetrics service at the Clinical Hospital of Pontificia Universidad Católica de Chile. Using western blot analysis, we evaluated phosphorylated and total protein levels of IRS-1, Akt, p42/44MAPK and eNOS in cells exposed or not to insulin. Isolated rings of umbilical veins were used to evaluate vasodilatation capacity by myography. The exposure (0-60 min) of HUVEC-N to physiological levels of insulin (1nM) showed a quickly and maintained increase of p~Akt and p~p44/42mapk. Conversely, HUVEC-OB showed a reduced and delayed p~Akt and p44/42mapk in response to insulin. Also, we found an increase in the inhibitory phosphorylation of IRS-1 and a reduced phosphorylation and total protein of eNOS, compared to HUVEC-N. Additionally, umbilical vein rings from MO showed less relaxation in response to insulin than rings from Normal pregnancies. In this study we have shown evidence that MO promotes neonatal IR in umbilical cord vein and endothelial cells.

200) Malin deficiency induces an upregulation of messenger RNA levesl of proteins involved in glycogen metabolism without modification in glycogen content and glycogen synthase activity in adult testis.

Villarroel-Espindola, F[1]., Duran, J[2].,Mancilla, H[1].,Maldonado, R[1].,Vander-Stelt, K[1].,Guinovart, J[2].,Slebe, J[1].,Concha, I[1].,[1]Bioquímica y Microbiología, Ciencias, Universidad Austral De Chile.[2]CIBER de Diabetes y Enfermedades Metabólicas (CIBERDEM) Institute for Research in Biomedicine (IRB Barcelona), España. (Sponsored by FONDECYT 3130449 And 1141033)

Glycogen synthesis is regulated by a complex mechanism. Laforin and malin are proteins able to indirectly regulate glycogen synthesis by proteasomal degradation of glycogen synthase (GS). Malin deficiency has been associated to the glycogen accumulation and apoptosis. We reported that the laforin-malin complex is functional in the seminiferous tubules. To understand the impact of the laforin-malin complex in adult testis we analyzed in a knockout model for malin protein (KO) the effects on glycogen homeostasis during spermatogenesis. Unexpectedly, the GS activity and stored glycogen in KO testis did not show differences compared to the control. In both, the histological studies reported normal spermatogenesis. By real time PCR analysis, relative levels of mRNAs involved in glycogen metabolism were upregulated in whole testis from KO mice, showing higher levels of mRNA for branching enzyme, glycogen debranching enzyme (AGL) and for the three isoforms of glycogen phosphorylase (GP), without affecting the levels of mRNA for GS. Additionally, in germ cells and Sertoli cells isolated from KO mice, both showed increased levels of mRNA for AGL and GP, specifically liver and brain isoform. These results suggest that malin deficiency could not impair spermatogenesis in adult testis by an unbalance on glycogen homeostasis and the upregulation of mRNA levels in testis could be a compensatory strategy to support any long-term effect by unclear mechanisms.

201) Participation of FoxO1 in cardiac fibroblast differentiation stimulated by TGF-β1

Vivar, R[1,2]., Díaz, G[2].,Chiong, M[2].,Lavandero, S[2,3].,[1]Química Toxicológica y Farmacológica, Ciencias Químicas y Farmacéuticas, Universidad de Chile.[2]Advanced Center for Chronic Diseases (ACCDiS) and Center for Molecular Studies of the Cell (CEMC), Ciencias Químicas y Farmacéuticas, Universidad de Chile.[3]Department of Internal Medicine, Southwestern Medical Center, University of Texas, Dallas. (This Work Was Supported By Post-Doctoral FONDECYT Grant 3130657 (to R.V.), FONDECYT 1130300 Grant (to G.D.A) And FONDAP 15130011 (to S.L, M.Ch And G.D.A.))

In cardiac fibrosis development is essential an active cardiac fibroblast (CF) differentiation to myofibroblast. TGF-β1 is a profibrotic factor involved in CF differentiation. The transcription factor FoxO1 mediates TGF-β1 action in various biological processes. Our aim was to investigate whether FoxO1 mediate TGF-β1-dependent CF differentiation in cultured neonatal CF. Our results showed that TGF-β1 treatment (10 ng/mL, 48 h) increased the protein levels of collagen I, CTGF, p21cip and α-SMA (assessed by Westernblot). Additionally, the cytostatic effect of TGF-β1 was evaluated using propidium iodide and flow cytometry. Our data showed that TGF-β1 decreased CF proliferation stimulated by FBS. Importantly, TGF-β1 increased protein levels of FoxO1 in concentration and time-dependent manner, this effect was prevented by actinomycin D and cycloheximide. The overexpression of FoxO1 in CF transduced with adenovirus resulted in higher expression of profibrotic factors and increased antiproliferative effect. The down regulation of FoxO1 levels with specific siRNAS, prevent the increase of profibrotic factors induced by TGF-β1 but not the CF proliferation stimulated by FBS. We concluded that TGF-β1 up-regulates and activates FoxO1 in CF and this transcription factor is required to induce TGF-β1-dependent CF differentiation

202) Adenosine regulates insulin-induced glucose uptake in cardiomyocytes

Westermeier, F[1]., Vásquez-Trincado, C[1].,Riquelme, J[1].,Valladares, D[1].,Jaimovich, E[2].,Lavandero, S[1,3].,[1]ACCDiS, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad De Chile.[2]Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad De Chile.[3]Dallas, Texas UT Southwestern Medical Center. (Supported By FONDAP 15130011 (SL), Anillo ACT1111 (SL & EJ), FONDECYT 1120212 (SL), FONDECYT 3140532 (EJ), FONDECYT 3140532 (FW), FONDECYT 3140491 (DV).)

The purine nucleoside adenosine plays a pivotal role in cardioprotectionimproving the cellular energy by activating four adenosine receptors (ARs) sub-types (A_1, A_{2A}, A_{2B}, and A_3). Insulin regulates cardiac metabolism by modulating lipid metabolism, protein synthesis, contractility and glucose transport in cardiomyocytes. However, despite the evidence described, a potential functional link between adenosine and insulin signaling pathways in the heart remains unclear. In this work, we analyzed the expression patterns for ARs in cultured rat cardiomyocytes. We observed that A_{2B}>A_{2A}>A_1 ARs were predominant compared with A_3 AR by quantitative PCR and Western blot assays. Moreover, A_{2B},A_{2A} andA_1 ARs exhibited a predominant cell surface localization using immunofluorescence microscopy. Interestingly, insulin-induced glucose uptake was blocked by caffeine (nonselective ARs antagonist) and ZM-241385 (A_{2A} antagonist) to values observed in absence of insulin. We conclude that adenosine trough activation of A_{2A} AR could modulate insulin signaling in the heart.

203) Cold-adapted enzyme from the ADP-dependent sugar kinase family: Effect of temperature on ligands selectivity.

Zamora, R[1]., Castro-Fernandez, V[1].,Guixé, V[1].,[1]Departamento de Biologia, Facultad de Ciencias, Universidad De Chile. (Sponsored by Fondecyt 1110137)

The enzymes from psychrophilic organism have evolved to perform its catalytic activity at low temperatures and this capability is attributed to their high structural flexibility. Also, many cold- adapted enzymes required a metal as a cofactor. This is case for members of the family of ADP-dependent kinases that used in Mg^{+2} as cofactor. In this work, we characterized kinetically the bifunctional cold-adapted enzyme phosphofructokinase/glucokinase from *Methanococcoides burtonii* (*Mb*PFK-GK). Interestingly, we found that the main activity of the enzyme (phosphofructokinase activity, PFK) can use different metals as cofactors depending on the temperature of the assay. At 10 °C *Mb*PFK-GK has higher activity with Co^{2+} whereas at 25 °C the higher activity was obtained in the presence of Mg^{2+}. Besides, sugar selectivity is also affected by temperature. At 10 °C *Mb*PFK-GK is only able to use glucose and fructose-6-phosphate as substrates, with no significant activity with other sugars. However, at 25 °C the enzyme is capable of use monosaccharides and disaccharides as substrates. These results highlighted the plasticity of this type of enzymes and pointed out the increase on flexibility as a key aspect of adaptation to low environmental temperatures.

204) MicroRNA signatures in breast cancer tumors with differential BRCA1 expression.

Zavala, V[1]., Alvarez, C[1].,Gamboa, J[2].,Cornejo, V[2].,Fernandez, W[2].,Carvallo, P[1].,[1]Biología Celular y Molecular, Ciencias Biológicas, Pontificia Universidad Católica De Chile.[2]., ., Hospital Clínico San Borja Arriarán. (Sponsored by FONDECYT1120200, CONICYT)

Cancer development is the consequence of the disturbance of the normal function of protooncogenes, tumor suppressor genes and their direct regulators, microRNAs (miRNAs). Deregulated miRNAs have been found in many cancer types, becoming an interesting target for cancer diagnosis and treatment. Several miRNAs can regulate BRCA1 expression, the main susceptibility gene for breast cancer development. Our aim is to analyze miRNA expression from fresh frozen breast cancer tumors, through the Agilent microRNA microarray system, and according tumor BRCA1 status. So far, we have evaluated 21 breast cancer tumor samples and compared their miRNA profiles with two normal breast tissue samples and one normal breast cell line. We found 49 significantly deregulated miRNAs in tumor samples relative to normal tissues. Thirty one are upregulated in more than 50% of tumors. Among them, ten have been validated *in vitro* or predicted by *in silico* approaches as BRCA1 regulators. Based on miRNA expression data bases for breast cancer tumors we selected two microRNAs as potential BRCA1 regulators in breast cancer. Hierarchical clustering analysis showed two major clusters for tumors, segregating separately from normal breast tissue and the cell line. Further analysis showed that these miRNAs are implicated in several cancer associated pathways, such as DNA damage-repair, transcriptional deregulation and oncogenic signaling pathways. FONDECYT1120200, CONICYT

205) Heterologous expression of a gene encoding a potential endo-ß-1,4-galactanase from *Penicillium purpurogenum*: purification and characterization of the recombinant enzyme.

Zavaleta, V[1]., Eyzaguirre, J[1].,[1]Ciencias Biológicas Universidad Andrés Bello. (Sponsored by FONDECYT 110084 And 1130180, UNAB DI-478-14/R And DI-73-12/I.)

Pectin of cell wall of plants is a polysaccharide rich in galacturonic acid. Among its components is type I rhamnogalacturonan (RGI). RGI is a chain with alternating galacturonic acid and rhamnose residues. The latter residue is substituted by galactose chains linked by β-(1,4), known as β-1,4-galactans. In turn, the β-1,4-galactans can be substituted with arabinose residues constituting arabinogalactans. These galactose polysaccharides are degraded by endogalactanases, which in the industry may have various applications, such as the production of probiotics, gelling agents and bioethanol. We identified a gene encoding a potential endo-ß-1,4-galactanase (1154 bp; including a 86 bp intron) from *Penicillium purpurogenum*, a saprophytic fungus that grows on a variety of carbon sources, among them sugar beet pulp. The objective of this work is to heterologously express and characterize this possible endo-β-1,4-galactanase. To this end, cDNA of the gene was obtained and cloned into the vector pPICZB, downstream of a methanol-inducible promoter. The cDNA was heterologously expressed in the yeast *Pichia pastoris* GS115 and the recombinant enzyme was purified by nickel-nitrilotriacetic acid resin (Ni-NTA). The enzyme has a molecular weight of 40 000 as determined by SDS-PAGE, which is similar to the theoretical value. The enzyme is active against lupine galactan. The optimal pH of the enzyme is from 2.5 to 6, and the optimum temperature is 50 – 60°C.

206) Galectin 8 and MCF7 cell conditioned media induce increased microvascular permeability via nitric oxide and S-nitrosation of p120 catenin

Zamorano, P[1]., Rebolledo, L[1].,Guequen, A[1].,Ross, B[2].,Mardones, G[2].,González, L[3].,Ehrenfeld, I[4].,Sosa, A[5].,Sanchez, F[1].,[1]Instituto de Inmunología, Facultad de Medicina, Universidad Austral De Chile.[2]Instituto de Fisiología, Facultad de Medicina, Universidad Austral De Chile.[3]Instituto de Reumatología, Facultad de Medicina, Pontificia Universidad Católica De Chile.[4]Instituto de Histología y Patología, Facultad de Medicina, Universidad Austral De Chile.[5]Intituto de Reumatología, Facultad de Medicina, Pontificia Universidad Católica De Chile. (Sponsored by FONDECYT 1130769)

A key characteristic of the tumor blood vessel is its increased permeability which ensures the delivery of oxygen and nutrients for expansion of malignant cells and maintenance of the cancer stem cell reservoir. We have shown that increased permeability in response to pro-inflammatory agents is linked to eNOS activation and S-nitrosation of b-catenin and p120. Galectin-8 (Gal-8) is widely expressed in tumor; promoting endothelial cell migration and angiogenesis. Since Gal-8 activates PI3-kinase-Akt pathway and this pathway is involved in eNOS activation, we tested the hypothesis that Gal-8 acts on the endothelium increasing microvascular permeability via nitric oxide and S-nitrosation of adherens junction proteins such us p120.

As a model system we use EA.hy926 cells treated with Gal-8. Permeability was measured through flux of dextran-FITC-70 in cellular monolayers. eNOS activation was evaluated through Western-blot and S-nitrosation of p120 was measured by biotin-switch assay.

Gal-8 increases permeability in EA.hy926 cells, induces eNOS phosphorylation and S-nitrosation of p120. Additionally we demonstrate that conditioned media from breast cancer cells (MCF7-CM) (which contains Gal-8) also increases permeability which is inhibited in the presence of L-NMA and lactose. This media also induces S-nitrosation of p120.

These results provide a new mechanism of action of Gal-8 through eNOS activation on the endothelial cells that can help to the progress of the tumor.

207) Effect of chronic Stress on Growth Hormone (GH)/Insulin like-Growth Factor 1 (IGF1) system in fish skeletal muscle growth.

Zuloaga, R[1]., Valenzuela, C[1].,Fuentes, E[1].,Estrada, J[2].,Valdes, J[1].,Molina, A[1].,[1]Biotecnologia Molecular, Ciencias Biologicas. INCAR (Centro Interdisciplinario para la Investigación Acuícola), Universidad Andrés Bello.[2]Centro de Investigaciones Marina Quintay (CIMARQ) Universidad Andrés Bello. (Sponsored by FONDECYT 1130545 And FONDAP INCAR 15110027)

Introduction: In mammals acute *stress* inhibits muscle growth and promotes atrophy. However, little is known about the effects of chronic *stress* and the participation of the endocrine system on this process. We assessed the effect of chronic *stress* on the GH/IGF system, which is the main responsible for muscle growth.

Methods: Fine flounder (*P. adspersus*) were subjected to 4 and 7 weeks of confinement inducing chronic *stress*. Cortisol plasma levels were measured by ELISA, the transcriptional regulation of the whole GH/IGF system by real-time PCR and the signaling pathway activation by western blot. Morphometric analyses of weight and size also were performed.

Results: Highcortisol levels were observed at 4 weeks of confinement, while a significant loss weight and weight/size ratio at 7 weeks. The *ghrs* expressions were differentially regulated at 4 weeks and the activation of JAK2/STAT5 was decreased in both times. Nonce, the *igf1* and *igf1r* expression were downregulated at 4 weeks, a dynamical expression was observed by the *igfbps* and a decrease in AKT/TOR/P70S6K/4E-BP1 activation at 7 weeks. Finally, a remarkable decrease of *myhc* expression was visualized at both times. **Conclusion**: These results show for the first time the effects of chronic *stress* on the GH/IGF1 system in fish. These data suggest that chronic *stress* directly affects muscle growth promoting atrophy by decreasing the GH/IGF1 system signal transduction.

208) New insights into the role of lysine-274 in the catalytic activity of Fructose 1,6-bisphosphatase

Asenjo, J[1]., Rivera, L[1].,Schott, S[1].,Ludwig, H[1].,Slebe, J[1].,[1]Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral De Chile.

FBPase, a key gluconeogenic enzyme, is inhibited by AMP, Fru2,6P_2 and high levels of substrate. We have demonstrated that binding of the substrate induces a second class of sites with lowered affinity and catalytic activity. Moreover, it has been postulated that the active site residue K274 is a binding site determinant for sugar bisphosphates and is essential for Fru2,6P_2 inhibition. However, the precise role of K274 in substrate/inhibitor binding and catalysis remains uncertain. Three types of experiments were used to shed light on this: (1) effect of Fru2,6P_2 on kinetic measurements over a very wide range of substrate concentration using wild-type and K274A FBPases; (2) fluorescence studies of a K274W mutant; and (3) kinetic studies of hybrid forms of the enzyme containing subunits in which K274 was replaced by alanine. The results show that in both mutants the K_M values are approximately four times higher than the value of the wild-type enzyme, while the kcat value of the K274A mutant increases and that of the K274W mutant decreases. Interestingly, in both mutants the inhibition by excess of substrate is drastically diminished whereas the Fru2,6P_2 inhibition changes only slightly. Unexpectedly, the K274W mutant senses the binding of AMP to the allosteric site but not the binding of the sugar bisphosphates to the active site. Overall, the data indicate that although K274 is an important determinant for sugar bisphosphates, it plays a more significant role in the mechanism of inhibition by excess of substrate. FONDECYT 1141033; DID-UACh 2013-45

209) Expert analysis of vehicles involved in criminal acts looking-for useful genetic fingerprints for human identification

Henríquez S[1]. , Rivera P[1]. , Alonso M[1], Cádiz R[1]. [1]Asesores Genética Forense Laboratorio de Genética Forense Departamento de Criminalística de Carabineros de Chile. [2]Director Técnico Laboratorio de Genética Forense Departamento de Criminalística de Carabineros de Chile. (Sponsored by Dra Lorena Norambuena)

In seeking to solve complex criminal cases in which vehicles recover as elements of committing them, we have developed numerous experiments to determine the best samples for obtaining a useful genetic fingerprint for comparison, in order to verify scientifically the suspects involvement arising from the police investigation.

We study the results obtained from real c ases and we analyze several undoubted samples from vehicles driven by different people, detecting that there are numerous factors that influence positive outcomes.

INDEX

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

Y

Z

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