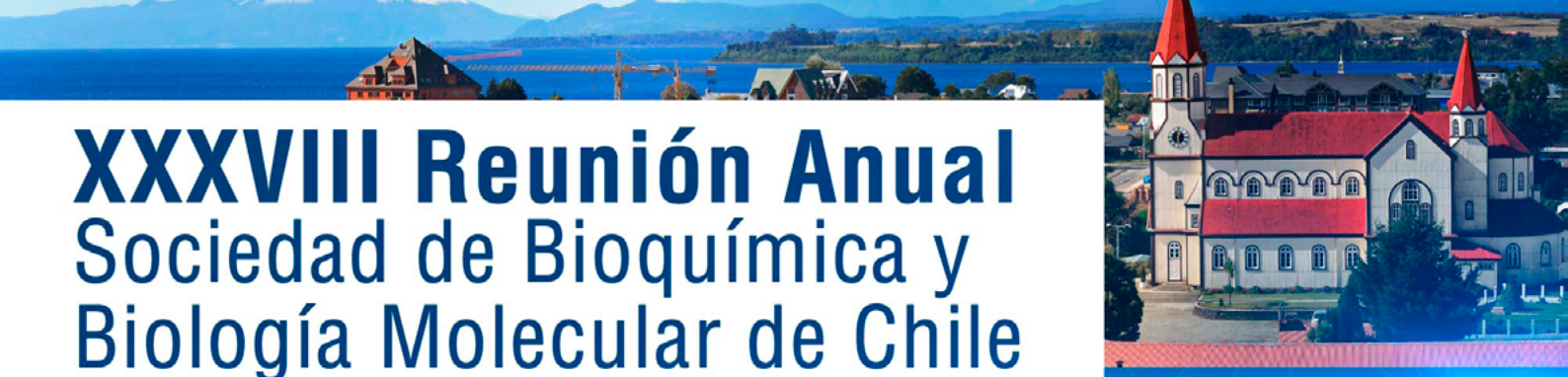

XXXVIII Reunión Anual
Sociedad de Bioquímica y Biología Molecular de Chile

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XXXVIII Annual Meeting
Sociedad de Bioquímica y Biología Molecular de Chile

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LECTURES

Opening Lecture

Neuroprotective effects of angiogenin-induced tRNA cleavage

Ivanov, Pavel[3]., O´Day, Elizabeth[1]., Emara, Mohamed[3]., Leiberman, Judy[1]., Wagner, Gerhard[2]., **Anderson, Paul[3]**., [1]Immune Disease Institute Harvard Medical School, Boston, MA 02115, USA. [2]Department of Biological Chemistry and Molecular Pharmacology Harvard Medical School, Boston, MA 02115, USA. [3]Division of Rheumatology, Immunology, and Allergy Brigham and Women´s Hospital, Boston, MA 02115, USA.

Angiogenin (ANG) is a stress-activated ribonuclease that promotes the survival of motor neurons. Ribonuclease inactivating point mutations are found in a subset of patients with amyotrophic lateral sclerosis. We recently showed that ANG cleaves tRNA within anticodon loops to produce 5'- and 3'-fragments known as tiRNAs. Selected 5'-tiRNAs (e.g., tiRNA^{Ala} and tiRNA^{Cys}) co-operate with the translational repressor YB-1 to displace eIF4F from m^{7}G-capped mRNA, inhibit translation initiation and induce the assembly of stress granules (SGs). Here we show that tiRNA^{Ala} binds the cold shock domain of YB-1 to activate these translational reprogramming events. We discovered that 5'-tiDNA^{Ala} (the DNA equivalent of 5'-tiRNA^{Ala}) is a stable tiRNA analogue that displaces eIF4F from capped mRNA, inhibits translation initiation, and induces the assembly of stress granules. 5'-tiDNA^{Ala} assembles a G-quadruplex structure that allows it to spontaneously enter primary motor neurons and trigger a neuroprotective response. Our results introduce 5'-tiDNA^{Ala} as a lead compound for the development of a new class of neuroprotective drugs.

Osvaldo Cori Lecture

The origins of bioenergetics

Vicuna, R[1]., [1]Genética Molecular y Microbiología, Ciencias Biológicas, Pontificia Universidad Católica De Chile.

Bioenergetics, perhaps the most characteristic feature of life, was one of Osvaldo Cori's favorite topics of interest. It is well known that all known forms of extant life rely on chemiosmotic proton (or sodium ion) gradients for the synthesis of ATP, heat production, flagellar rotation or active transport across membranes. The universality of this mechanism suggests that it evolved early on. If this was indeed the case, how did primitive life manage something that today requires highly sophisticated molecular machinery? Proton gradients form naturally at alkaline hydrothermal vents on the sea floor. They are formed as seawater percolates down into electron dense rocks found in the Earth´s mantle, such as the iron-magnesium mineral olivine. Olivine reacts with water to produce serpentinite, hydroxides, hydrogen gas and low molecular weight hydrocarbons. When the alkaline serpentinization effluents come into contact with the cooler acidic ocean waters, minerals precipitate out forming porous towering vents. The geologist Michael Russell proposed several years ago that the earliest cells may have harnessed this geochemically created proton-motive force to convert CO_2 into organic molecules inside the cell-like spaces of the porous vents. Minerals present in the "cell" walls played a crucial role catalyzing these reactions. According to Russell, when cells learned to make their own gradients, they were able to leave the vents. During the lecture, arguments supporting Russell's hypothesis will be discussed.

Severo Ochoa Lecture

RNA as a modulator of genome dynamics and chromatin structure

Aguilera, Andres[1].,[1]Molecular BIology, CABIMER, Sevilla.

Nascent eukaryotic mRNA is assembled into a nuclear ribonucleoprotein complex that is processed and exported to the cytoplasm for its translation. However, in particular cases, RNA can form structures, such as R-loops, made of RNA-DNA hybrids and the displaced single-stranded DNA, with a different fate. These structures may have important consequences in genome stability as well as in the modulation of chromatin structure. Although, recent reports have shown that R-loop-mediated changes in chromatin can have a positive role in eukaryotes, evidence shows that R-loops may be an important source of genome instability. We have shown that this may constitute a relevant cause of transcription-associated genome instability, in large part due to its ability to impair replication fork progression. Although the mechanism responsible for R-loop formation and how it leads to genome instability is yet unclear, we have evidence that a major cause for this phenomenon is the ability of R loops to trigger chromatin compaction. I will discuss our recent results in yeast and human cells about the involvement of the nascent RNA in modulating genome dynamics and chromatin structure, the possible mechanisms to explain these phenomena, and the implications of tumor suppressor genes in preventing R-loop accumulation and R-loop-mediated genome instability.

PABMB Lecture

Spliceosome and mRNP Structure Function and Dynamics

Moore, Melissa J.

Melissa.Moore@umassmed.edu

In cells, all RNA molecules interact with proteins to form ribonucleoprotein particles (RNPs). Some of these particles are highly stable (e.g., ribosomes), while others are subject to dynamic assembly, rearrangement and disassembly. Research in my laboratory focuses on understanding the machinations of two highly complex RNPs: spliceosomes and messenger RNPs. Spliceosomes are the macromolecular machines responsible for recognizing and removing introns from nascent transcripts. Consisting of five small nuclear RNAs (snRNA) and scores of attendant proteins, these complexes must reassemble from separate pieces on each new intron, undergo complicated rearrangements to become activated for catalysis, and then once again disassemble when splicing is complete. Together with the capping and polyadenylation machineries, spliceosomes produce mRNAs, which are associated with scores of other proteins to form mRNPs. Mature mRNPs are transported to the cytoplasm where they undergo regulated localization, translation and decay. All of these processes are positively and negatively modulated by mRNP proteins, many of which come and go as the mRNA proceeds through its lifecycle. We use next generation sequencing and single molecule approaches to investigate many different questions related to large RNP structure, dynamics and function. Recent progress includes identification of multiple pathways for spliceosome assembly, new insight into overall mRNP architecture, and direct demonstration of active translation by 80S monosomes.

SYMPOSIA

SYMPOSIUM 1
ACCDIS SYMPOSIUM ON MOLECULAR MECHANISMS OF CHRONIC DISEASES

Chairs: Lorena García, Mario Chiong, Universidad de Chile

Smooth muscle cytoskeleton regulation in Vascular Biology

San Martin, Alejandra, Emory University School of Medicine, Atlanta, Georgia, USA.

In vascular smooth muscle cells (VSMCs), cytoskeleton dynamics impact vascular diseases through the regulation of cell growth, migration, tension generation and stiffness. Recent work has shown that reactive oxygen species (ROS) are important signaling mediators responsible for cytoskeletal integrity and reorganization. NADPH oxidases are major sources of reactive oxygen species (ROS) in the vasculature. In fact, VSMCs from large arteries express two homologues of NADPH oxidases, Nox1 and Nox4 and its regulatory protein Poldip2. Our laboratory has described the function and mechanism of Nox-dependent pathways that regulate lamellipodia formation and cell adhesion during migration and the cellular responses to mechanical strain. Because of the cytoskeleton is not only key during migration but fundamental during intracellular compartment movement, recently we have become interested in the participation of Poldip2 in mitochondrial dynamics. We believe that Nox-mediated regulation of the cytoskeleton has a key role in the maintenance of vessel homeostasis and, when deregulated, contribute to vascular diseases. Therefore, understanding the redox-sensitive pathways that contribute to cytoskeleton reorganization may facilitate the identification of therapeutic targets.

Preclinical Studies for Sodium Tungstate and its effect in diabetic nephropathy.

Yañez, Alejandro[2]., Bertinat, Romina[1].,Jaramillo, Karen[2].,Silva, Pamela[2].,Carpio, Juan Daniel[3].,Slebe, Juan Carlos[2].,[1]Centro Microscopia Avanzada (CMA) Bio-Bio Universidad De Concepción.[2]Instituto de Bioquimica y Microbiologia, Facultad de Ciencias, Universidad Austral De Chile.[3]Instituto de Anatomía, Histología y Patología, Facultad de Medicina, Universidad Austral De Chile. (Sponsored by FONDECYT 1131033)

The condition of constant hyperglycemia patients suffering from diabetes is the main generator of micro and macro-vascular damage in peripheral tissues, resulting in the increase in the amount of pathologies associated with the disease. One of these conditions is diabetic nephropathy, the leading cause of ESRD in the world, where processes such as fibrosis, inflammation and oxidative stress, causing cellular damage and the following reduction of renal function. In spite of the available therapies of glycaemic and blood pressure control, many patients continue to show progressive renal damage. Therefore it is very important to identify new therapeutic interventions to halt the progression of DN. Studies from our laboratory have shown that sodium tungstate (NaW) causes normalization of glycaemia, glycosuria and albuminuria levels in models of streptozotocin-induced diabetic rats. The anatomopathological analysis showed that structural alterations of the kidney as glomerular lesions were reduced by 70% and tubular damage decreased 50%. Moreover, NaW treatment inhibited the NF-κB activity and markedly decreased ICAM-1, MCP-1 and TGF-β1 protein expression in kidney. It was also found reduced expression of α -SMA which correlates with decreased renal fibrosis. The effect of this drug is able to reverse these processes at the cellular level by inhibiting oxidative stress, and decreasing inflammation and fibrosis, which are closely linked to the generation of renal damage during development of diabetic nephropathy. These results reveal some of the mechanisms induced by NaW in the reversion of diabetic nephropathy, and propose its potential application in the treatment of this disease.

Metabolic regulation of vascular smooth muscle cell dedifferentiation

Chiong, M[1]., Cartes-Saavedra, B[1].,Morales, P[1].,Torres, G[1].,Norambuena-Soto, I[1].,Mondaca-Ruff, D[1].,Sanhueza-Olivares, F[1].,Garcia-Miguel, M[1].,[1]ACCDiS, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad De Chile. (Sponsored by Fondecyt 1140329, FONDAP 15130011, Anillo ACT1111.)

Differentiation and dedifferentiation of vascular smooth muscle cells (VSMCs) play a critical role in the pathogenesis of vascular diseases, such as atherosclerosis, hypertension and vascular stenosis. We hypothesize that VSMC phenotypic switching is regulated by cell metabolism, in particular mitochondrial function and autophagy. Mitochondrial function can be increased by mitochondria-endoplasmic reticulum coupling and mitochondrial fusion. Prevention of both processes inhibits VSMC dedifferentiation induced by platelet-derived growth factor-BB (PDGF-BB). Moreover, PDGF-BB, Ang II and TNF-α induce VSMC dedifferentiation and autophagy. In PDGF-BB-treated VSMCs, autophagy is associated with a reduction in the number of mitochondria. Inhibition of autophagy completely prevents VSMC dedifferentiation. Taking together, our data suggest that cell metabolism is an important regulator of VSMC phenotype. Regulation of VSMC metabolism could be a new target in the treatment of vascular diseases.

VCAM-1: a novel therapeutic target and biomarker for cardiac diseases

García, Lorena
Advanced Center for Chronic Diseases (ACCDiS), Facultad Ciencias Químicas y Farmacéuticas, Universidad de Chile. E-mail: logarcia@ciq.uchile.cl

Vascular cellular adhesion molecule-1 (VCAM-1) is a well-known cell adhesion protein of the immunoglobulin superfamily involved in inflammation. VCAM-1 mediates the adhesion of lymphocytes and monocytes to vascular endothelium and plays a key role in the development of atherosclerosis. However this protein has also non-immune functions such as in the development of the heart mice. Soluble VCAM-1 (sVCAM) has emerged as biomarker for cardiac inflammation and endothelial activation.

On the other hand, post-operative atrial fibrillation (POAF) is a common complication after coronary artery bypass surgery affecting approximately 30% of the patients. Our group evaluated the role of several systemic and tissue biomarkers, including VCAM-1 and the intercellular adhesion molecule-1 (ICAM1) in order to identify patients with a susceptible cardiac substrate that render them prone to the development of POAF. Our main finding in this study was that baseline circulating levels of sVCAM-1 predicts the occurrence of POAF following coronary artery bypass surgery in coronary patients in normal sinus rhythm prior to surgery.

Interestingly, our recent work shows that VCAM-1 is also expressed in rat cardiomyocytes and required to maintain insulin-dependent cardiomyocyte viability. Currently we are studying how the protective effect of insulin on myocardial ischemia is lost when decrease cardiomyocyte VCAM-1 levels.

FONDECYT 1070641 & 1110346, FONDAP 15130011.

SYMPOSIUM 2
MICROBIAL PATHOGENESIS
Chair: Luis F. Larrondo, P. Universidad Católica de Chile

Genome-wide identification of genes required for *Salmonella* to survive within the host

Santiviago, C[1]., [1]Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad De Chile.

Two libraries of individual single gene deletion (SGD) mutants of *Salmonella enterica* serovar Typhimurium strain 14028s encompassing deletions of 3,923 annotated non-essential ORFs and sRNAs were screened by intraperitoneal (IP) injection in BALB/c mice followed by recovery from spleen and liver two days post infection. The relative abundance of each mutant was measured by microarray hybridization. The two mutant libraries differed in the orientation of the antibiotic resistance cassettes (either sense-oriented Kan^R or antisense-oriented Cm^R). Consistent systemic colonization defects were observed in both libraries and both organs for hundreds of mutants of genes previously reported to be important after IP injection in this animal model, and for about 100 new candidate genes required for systemic colonization. Four mutants with a range of apparent fitness defects were confirmed using competitive infections with wild-type parental strain: Δ*STM0286*, Δ*STM0551*, Δ*STM2363* and Δ*STM3356*. Two mutants, Δ*STM0286* and Δ*STM2363*, were then complemented in *trans* with a plasmid encoding an intact copy of the corresponding wild-type gene, and regained the ability to fully colonize BALB/c mice systemically. These results suggest the presence of many more undiscovered *Salmonella* genes with phenotypes in IP infection of BALB/c mice and, more importantly, validate the libraries for application to other systems. Currently, we are screening our SGD libraries to identify genes required for intracellular survival of *S*. Typhimurium in murine macrophages and protozoa, using the social amoeba *Dictyostelium discoideum* as host model.

This work was supported by FONDECYT grants 1110172 and 1140754.

Role of an autophagy regulator in the pathogenesis of *Cryptococcus neoformans*

Rodrigues, M[1,2]., [1]Center for Technological Development in Health (CDTS) Oswaldo Cruz Foundation - Fiocruz. [2]Microbiology Institute Federal University of Rio de Janeiro. (Sponsored by This Work Was Supported By CNPq, CAPES, FAPERJ And Instituto Nacional De Ciência E Tecnologia De Inovação Em Doenças Negligenciadas (INCT-IDN). MR Is The Recipient Of A Pathfinder Award From The Wellcome Trust (UK))

Unconventional secretory activity is essential for virulence mechanisms of *Cryptococcus neoformans*, a fungal pathogen responsible for 500,000 deaths each year in the globe. Autophagy, a basic mechanism required for degradation of cellular components, participates in unconventional secretion pathways in Eukaryotes. On the basis of the connections between unconventional secretory mechanisms and fungal virulence, we investigated the involvement of the autophagy regulator Atg7p in physiology and pathogenic potential of *C. neoformans*. Deletion of *C. neoformans ATG7* resulted in marked alterations in the vesicular export of small RNA. The *atg7Δ* mutant also manifested altered distribution of surface components, high sensitivity to the standard antifungal amphotericin B and increased cellular size both *in vitro* and *in vivo*. In comparison to wild type and complemented cells, the *atg7Δ* mutant had decreased survival in the lung of infected mice, higher susceptibility to the antifungal arsenal of different host phagocytes and reduced ability to kill an invertebrate host. In summary, these results connect autophagy regulation and fungal pathogenicity in the *C. neoformans* model.

Bacterial Warfare: A new role for the Type IV Secretion System

Souza, Diorge[1]., Oka, Oka[1]., German, Sgro[1]., Oliveira, Luciana[1]., Bueno, Natalia[1]., Favaro, Denize[1]., Salinas, Roberto[1]., **Farah, C[1]**., [1]Departamento de Bioquímica Universidade de São Paulo. (Sponsored by Financial Support: FAPESP And CNPq)

Type IV secretion systems (T4SSs) are multiprotein complexes that transport effector proteins and protein–DNA complexes through bacterial membranes to the extracellular milieu or directly into the cytoplasm of other cells. Many bacteria of the family Xanthomonadaceae, which occupy diverse environmental niches, carry a T4SS with several characteristics that distinguishes it from other T4SSs. Here we show that the Xanthomonas citri T4SS provides these cells the capacity to kill other Gram-negative bacterial species in a contact-dependent manner. The secretion of one type IV bacterial effector protein is shown to require a conserved C-terminal domain and its bacteriolytic activity is neutralized by a cognate immunity protein whose 3D structure is similar to peptidoglycan hydrolase inhibitors. This is the first demonstration of the involvement of a T4SS in bacterial killing and points to this special class of T4SS as a mediator of both antagonistic and cooperative interbacterial interactions.

Employing *Botrytis cinerea*, *Arabidopsis thaliana* and *Solanum lycopersicum* to understand environmental prompts as modifiers of the host-pathogen interaction

Canessa, P[1]., Hevia, Montserrat[1]., Muller-Esparza, Hanna[1]., Vega, Andrea[2]., Larrondo, Luis[1]., [1]Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile. [2]Departamento de Ciencias Vegetales, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica De Chile. (Sponsored by Fondecyt 11140678 And Núcleo Milenio De Biología Fúngica Integrativa Y Sintética NC120043.)

Organisms are surrounded by both abiotic and biotic signals that strongly modify their behavior. Although these cues are generally analyzed as single isolatedvariables, in this work we present data of environmental signals that modulate the interaction between the important phytopathogenic fungus - *Botrytis cinerea* - and two plant hosts. First, by employing *A. thaliana* and a bluelight blind *B. cinerea* mutant, we demonstrate the effect of light on virulence and differentiation on this phytopathogen. Unpublished genome-wide expression data supports the presence of light-mediated transcriptional responses in Botrytis that are more complex than those described in other wellstudied fungal models. Second, and also employing Arabidopsis, we analyzed the impact of time, an often underestimated environmental variable, in the plant-pathogen interaction. The data supports the notion of a time-of-the-day dependent plant-pathogen interaction. Finally, and also employing genome-wide expression data, we analyze the effect on nitrogen on both *B. cinerea* developmental programs and in its interaction when infecting *S. lycopersicum*. Future work and putative connections will be discussed. Funding: Fondecyt 11140678 and Núcleo Milenio de Biología Fúngica Integrativa y Sintética NC120043.

SYMPOSIUM 3
CENTRAL DOGMA AT THE SINGLE MOLECULE LEVEL
Chair: Christian A.M. Wilson, Universidad de Chile

Transcription regulation through changes in the DNA - RNA polymerase contacts. *An AFM and optical-tweezers combined study.*

Guerra, Daniel[1]., Sosa, Robert[1]., Piere, Rodríguez-Aliaga[1,2]., Doniselli, Nicola[3]., Rivetti, Claudio[3]., Bustamante, Carlos[4]., [1]Laboratorio de Moléculas Individuales, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia. [2]Jason L. Choy Laboratory of Single Molecule Biophysics University of California - Berkeley. [3]Dipartimento di Bioscienze Università degli Studi di Parma. [4]Jason L. Choy Laboratory of Single Molecule Biophysics University of California, Berkeley.

The stringent response is a cellular mechanism by which bacteria rapidly inhibit the expression of genes related to growth and stimulate others related to stress survival. Currently, the observed changes are best explained by the differential regulation of transcription initiation for different promoters. In order to better understand how the interactions within the transcription initiation complexes (RPO) determine such differential regulation, we studied Escherichia coli RNA polymerase-sigma 70 holoenzyme (RNAP) in interaction with bacteriophage lambda pR or ribosomal promoters through single-molecule structural and dynamic approaches. AFM images showed different levels of DNA compaction corresponding to DNA wrapping on the surface of RNAP enzyme in a specific way for each kind of promoter. In the presence of the stringent-response modulators, ppGpp and DksA, the extent of DNA-protein contacts were reduced with consequences in the stability of the RPO, thus offering a new model for transcription regulation. The highly-stable wrapped conformation of the pR promoter was further characterized by reversible mechanical denaturation with optical tweezers. By holding and pulling a single DNA molecule between two polystyrene beads, we were able to observe a transition that corresponds to the partial and reversible dissociation of the protein-DNA interactions of RPO complexes. Importantly, the change in extension determined in this way is closely compatible to the extension of the compaction observed by AFM images. The kinetic and thermodynamic characterization of this system offers new insights on the energetics of transcription initiation as it is influenced by extended specific contacts between RNAP and enhancer DNA elements.

Mechanical Force Releases Nascent Chain-Mediated Ribosome Arrest In Vitro and In Vivo

Goldman, D[1]., Kaiser, Christian[2]., Milin, Anthony[2]., Righini, Maurizio[3]., Tinoco, Ignacio[1]., Bustamante, Carlos[4]., [1]Chemistry University of California-Berkeley. [2]Biology Johns Hopkins University. [3]QB3 University of California-Berkeley. [4]Physics, Chemistry, Molecular and Cell Biology University of California-Berkeley. (Sponsored by HHMI, NSF Graduate Research Fellowship, NIH 5R01GM32543 And NIHGM10840)

During translation elongation, the ribosome decodes the mRNA and synthesizes protein. Variations in elongation rate are important for gene expression and for proper folding and activity of the nascent protein. Such variations can be caused by interactions between the nascent chain and the ribosome exit tunnel, which stall translation. Using optical tweezers, we demonstrate that a force applied to the nascent polypeptide can release nascent-chain mediated arrest. Additionally, we show that a nascent protein folding near the ribosome exit tunnel can generate a force capable of restarting stalled ribosomes. We propose a kinetic model describing how a protein can modulate translation through the force generated by folding.

Mechano-Physiology of the Giant Muscle Protein Titin

Rivas Pardo, Jaime Andres[1]., Eckels, Edward [1]., Popa, Ionel[1]., Kosuri, Pallav[1]., Linke, Wolfgang[2]., Fernandez, Julio[1]., [1]Department of Biological Sciences Columbia University. [2]Department of Cardiovascular Physiology Ruhr University Bochum, New York, Germany. (Sponsored by This Work Was Supported By Deutsche Forschungsgemeinschaft Grant SFB1002/TPB03 (W.A.L.) And The National Institutes Of Health Grants HL66030, HL61228 And National Science Foundation Grant 1252857 (J.M.F.))

Titin is the largest protein in the human body. Constituted by Immunoglobulin-like (Ig) domains and unstructured regions, titin determines the passive elasticity of muscle tissue. Current theories of muscle contraction propose that the power stroke of a myosin motor is the sole source of mechanical energy driving the sliding filaments of a contracting muscle. These models exclude titin, which determines the passive elasticity of muscles. We have combined single-particle tracking and single-molecule force spectroscopy to observe the dynamics of Ig domains from I-band under physiological forces of 6-8 pN. Using quantum dots and centroid tracking techniques we show that in single myofibrils extended to an optimal sarcomere length of 3.1 µm, titin molecules labeled *in situ* undergo stepwise changes in length of 13 ± 3 and 22 ± 3 nm. Single-molecule experiments on a proximal native fragment of titin shows that the folding/unfolding step sizes of the proximal Ig domains of 10 ± 4 and 22 ± 5 nm at 6 pN of force, matching the step size observed in the intact single myofibril. Additionally, our results indicate that unfolded titin Ig domains undergo a spontaneous stepwise folding contraction at forces below 10 pN, delivering up to 105 zJ of additional contractile energy, which is larger than the mechanical energy delivered by the power stroke of a myosin motor. In conclusion, our studies demonstrate that Ig domain folding reactions in titin occur at physiological forces and sarcomere lengths and are thus likely to be a major component of muscle elasticity.

The ClpXP protease unfolds substrates using a constant rate of pulling but different gears

Maillard, R[1]., [1]Chemistry Georgetown University.

ATP-dependent proteases are vital for the maintenance of cellular protein homeostasis, but the mechanisms by which these machines couple ATP hydrolysis to mechanical protein unfolding and polypeptide translocation remain unclear. Here, we use optical tweezers to study the mechanism of intersubunit coordination in the ClpXP protease from *Escherichia coli*. We reveal that ClpXP translocates substrate polypeptides by highly coordinated conformational changes in up to four of its six ATPase subunits. To successfully unfold stable substrates like GFP, ClpXP must use this maximum successive firing capacity. Unraveling of GFP is cooperative and proceeds via two intermediate states. The dwell duration between individual bursts of polypeptide translocation is constant and governed by an internal clock, regardless of the number of translocating subunits. Based on these findings, we conclude that ClpXP operates with constant *rpm* but different *gears*.

SYMPOSIUM 4
EPIGENETIC AND CHROMATIN STRUCTURE: FROM CELL FUNCTION TO BIOMARKERS
Chair: Paola Casanello, P. Universidad Católica de Chile

TNFa mediated changes in chromatin structure during gene activation

Längst, G[1]., [1]Biochemistry University of Regensburg.

Background: The rearrangement of nucleosomes along the DNA fiber profoundly affects gene expression, but little is known about how signalling reshapes the chromatin landscape, in three-dimensional space and over time, to allow establishment of new transcriptional programs.
Results: Using micrococcal nuclease treatment and high-throughput sequencing, we map genome-wide changes in nucleosome positioning in primary human endothelial cells stimulated with tumour necrosis factor alpha (TNFα) - a proinflammatory cytokine that signals through nuclear factor kappa-B (NF-κB). Within 10 min, nucleosomes reposition at regions both proximal and distal to NF-κB binding sites, before the transcription factor quantitatively binds thereon. Similarly, in long TNFα-responsive genes, repositioning precedes transcription by pioneering elongating polymerases and appears to nucleate from intragenic enhancer clusters resembling super-enhancers. By 30 min, widespread repositioning throughout megabase pair-long chromosomal segments, with consequential effects on three-dimensional structure (detected using chromosome conformation capture), is seen. Conclusions: Whilst nucleosome repositioning is viewed as a local phenomenon, our results point to effects occurring over multiple scales. Here, we present data in support of a TNFα-induced priming mechanism, mostly independent of NF-κB binding and/or elongating RNA polymerases, leading to a plastic network of interactions that affects DNA accessibility over large domains.

Epigenetic control of cell fate.

Montecino, Martin[1]., [1]Center for Biomedical Research and FONDAP Center for Genome Regulation, Faculty of Biological Sciences and Faculty of Medicine, Universidad Andrés Bello. (Sponsored by FONDECYT 1130706 And FONDAP 15090007)

Multiple dimensions of epigenetic control contribute to regulation of gene expression during mammalian cell differentiation. In this presentation, the evidence presented supports the role of epigenetic mechanisms during regulation of transcription of critical genes for mesenchymal cell lineage commitment. Special attention is dedicated to discuss recent results leading to the identification of key epigenetic mechanisms that contribute to both silencing and activation of bone-master regulators during differentiation of mesenchymal uncommitted cell precursors to non-osteoblastic and osteoblastic cell types, respectively. Our results shed light on how chromatin-remodeling processes can be associated with the presence of defined patterns of post-translational modifications in histones H3 and H4 that are bound to the promoter region of phenotypic genes. Importantly, these data demonstrate that key members of the polycomb and trithorax group of transcriptional regulators as well as proteins mediating DNA methylation and de-methylation, are critical components during BMP2-dependent transcriptional activation of osteoblast-related genes.

Fetal programming of endothelial function by epigenetic mechanisms: interaction with nitric oxide.

[1]Bernardo J Krause, [2]Emilio Herrera, [1,3]Cherie Hernández, [1,3]Farah Díaz, [1,3]Andrés Caniuguir, [2]Cristián Villanueva, [2]Esteban Figueroa, [3]Ricardo Uauy, [1,3]Paola Casanello. [1]Division of Obstetrics and Gynecology, Division of Pediatrics, School of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile. [2]Laboratorio de Función y Reactividad Vascular, ICBM, Facultad de Medicina, Universidad de Chile, Santiago, Chile. [3]Division of Pediatrics, School of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile

Endothelial cells (EC) from Intrauterine Growth Restriction (IUGR) placentae present an altered expression of endothelial NO synthase (eNOS) and arginase-2 (Arg-2) affecting the vascular function. In normal EC eNOS expression is epigenetically defined by mechanisms which can be subsequently affect the NO-dependent control of histone acetylation. We studied whether IUGR-derived cells present an altered DNA methylation pattern in the promoter region of eNOS and ARG2 genes, and the effect of NO in the expression of these genes. Methods. Human umbilical endothelial cells (HUEC) were obtained from Normal (N) and IUGR placentae. Specific CpG methylation and histone 3 and 4 acetylation at *NOS3* and *ARG2* gene promoters were determined by pyrosequencing and ChIP respectively. Cells were treated with NOC-18 (NO donor) and DNMT knocked down using siDNMT to test the role of NO and DNMT1 on gene expression. Results. The altered expression of eNOS in IUGR associated with altered DNA methylation in the proximal promoter. No effect of methylation was observed on *ARG2* gene. The silencing of DNMT1 in IUGR HUEC restored to normal levels the expression of eNOS. Conversely NOC-18 induced arginase-2 and decreased eNOS gene expression, an effect that correlated with changes in chromatin accessibility and increased H3 and H4 acetylation at ARG2 gene promoter. Conclusions. Changes in eNOS expression in IUGR-HUEC associate with altered DNA methylation at the NOS3 proximal promoter which is preserved via DNMT1. In contrast arginase-2 expression is not associated with DNA methylation changes but is tightly controlled by NO in an epigenetic-dependent mode.
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Epigenetic alterations and potential biomarkers in digestive tumors

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Epigenetic factors, such as DNA methylation and microRNAs, are important regulators of gene expression. DNA methylation is a process in which cytosines acquire a methyl group in 5'-position only if they are followed by a guanine (CpG site) resulting in gene silencing. It has been suggested that infectious agents induce aberrant DNA methylation and dysregulation of microRNA expression. Moreover, DNA methylation can also be reversed by clearance of the infection. Therefore, epigenetic factors may be considered an unique opportunity to explain how environmental factors increase the susceptibility for cancer development. Specific DNA methylation and/or microRNAs alterations have been reported in digestive tumors, particularly in gastric cancer, one of the most common cancers in the world. Here, we will present current data that shows that these epigenetic alteratios are not only associated with cancer development but also that might be translated into potential biomarkers for early detection and monitoring disease. Specifically, through the measurement of circulating DNA (cell-free DNA; cfDNA) we and others have proposed several candidates that displays significative differences between cases and controls suggesting an opportunity for non-invasive detection of gastric cancer.

SYMPOSIUM 5
GETTING THE MESSAGE: THE COMPLEX LIFE OF EUKARYOTIC MRNAS
Chair: Ricardo Soto-Rifo, Universidad de Chile

HIV-1-mediated endolysosome translocation: Impact on viral RNA localization and host metabolism

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Mammalian target of rapamycin (mTOR) kinase couples metabolic and stress signals to pathways that mediate cellular growth and proliferation. mTOR maintains the activation of downstream effectors important for host cell metabolism including mRNA translation, cell proliferation and catabolic processes such as autophagy. mTOR activation requires late endosome/lysosome recruitment via Rag GTPases. In earlier work, we found that RagA co-precipitated with the HIV-1 RNP and in situ analyses revealed co-localization with mTOR in cytoplasmic punctae. Using confocal microscopy, we assessed the HIV-1-mediated efects of HIV-1 on mTOR. Oxidative stress mediated the predominant accumulation of mTOR to the perinuclear region, colocalizing with *lysosomal-associated membrane protein* 1 (LAMP1), but not with TIAR. In striking contrast, HIV-1 induced the peripheral distribution of LAMP1/mTOR and strikingly, RagA depletion impaired this. Phospho-S6K-1 and -4E-BP1 were induced to the same extent by oxidative stress in either mock or HIV-1-expressing cells. Finally, HIV-1 did not reverse mTOR inhibition in cells treated with mTOR inhibitors. These findings demonstrate that HIV-1 modulates mTOR activity and localization by two distinct mechanisms: one that targets an event upstream of mTOR and another that subverts viral trafficking, which in turn, seems to be dependent on the demonstrated interaction between HIV-1 and Rag-GTPase.

RIPiT-seq reveals the endogenous RNA-target sites of DEAD-box protein 3 (DDX3)

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DEAD-box proteins are RNA helicases involved in numerous aspects of RNA metabolism. DDX3, a member of this family, has been shown to regulate pre-mRNA splicing, nuclear export of mature mRNAs and translation. Furthermore it also acts as an essential host factor required for replication of several viruses. Interestingly, DEAD-box proteins interact with RNA in a sequence-independent manner through the phosphodiester backbone. Therefore understanding how such an unspecific interaction can lead to specific effects on gene regulation is of great interest. Here we propose to study the role of DDX3 in regulating gene expression through the mapping of its endogenous RNA-binding sites. For this, we have used a novel CRISPR protocol to introduce a Flag-tag at the endogenous DDX3 locus. This allowed us to efficiently use our RIPiT-seq procedure (RNA-Immunopurification in Tandem) without having to overexpress a recombinant form of DDX3 ectopically. We expect our approach to reveal the main structural characteristics defining a DDX3 binding site and provide a new platform for the study of RNA-binding proteins that avoids all biases induced by overexpressing the protein of interest.

Comprehensive identification of RNA-binding domains in vivo.

Castello, A[1]., Fischer, Bernd[2]., Christian, Frese[3]., Jeroen, Krijgsveld[3]., Hentze, Matthias[3]., [1]Biochemistry University of Oxford. [2]German Cancer Research Center (DKFZ) German Cancer Research Center (DKFZ). [3]Genome Biology European Molecular Biology Laboratory. (Sponsored by A.C. Is Supported By An MRC Career Development Award (#MR/L019434/1). M.W.H. Acknowledges Support By The ERC Advanced Grant ERC-2011-ADG_20110310 And By The Virtual Liver Network Of The German Ministry For Science And Education. CKF Is Supported By The EM)

The RNA interactomes of HeLa and HEK293 cells jointly comprise 1106 RNA-binding proteins (RBPs) (1, 2, 3). Almost half of these lack well-defined RNA-binding domains (RBDs), suggesting the existence of unknown modes of RNA binding. Here, we report RBDmap, a new method to comprehensively determine the protein domains engaged in RNA binding in living cells. Applying in vivo UV crosslinking, purification of polyadenylated RNAs on oligo (dT) beads, controlled proteolysis and mass spectrometry, RBDmap identified 1085 RNA-binding sites in 505 proteins. RBDmap not only re-discovered the well established RBDs (e.g. RRM, KH) , but also identified dozens of novel RNA-binding motifs across homologous and non-homologous proteins. Importantly, about half of the protein-RNA interactions reported here are mediated by low complexity, disordered motifs, implying a new dimension of intrinsically disordered proteins in RNA binding. RBDmap thus instructs on the modes of RNA-binding of hundreds of proteins in their native cellular states, providing valuable structural and functional insights into RNA biology. 1. A. G. Baltz et al., Mol Cell 46, 674 (2012). 2. A. Castello et al., Cell 149, 1393 (2012). 3. A. Castello et al., Nat Protoc 8, 491 (2013).

From the nucleus to the ribosome: understanding post-transcriptional control of the HIV-1 unspliced Mrna.

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Post-transcriptional control of Human Immunodeficiency Virus type-1 (HIV-1) gene expression is a highly regulated process that commences in the nucleus of the host infected cell and finished by the expression of viral proteins in the cytoplasm. Viral gene expression involves the synthesis of a complex transcriptome including a subset of completely and incompletely spliced transcripts as well as one unspliced mRNA. Similar to cellular mRNAs, completely spliced transcripts follow the classical gene expression pathway in which intron removal during splicing strongly stimulates the rates of nuclear export and translation through the recruitment of different adaptor proteins such as SR proteins and the exon junction complex (EJC). Although unspliced mRNAs are normally retained and degraded in the nucleus of eukaryotic cells, expression of the HIV-1 unspliced mRNA is particularly controlled at the level of nuclear export and translation. During this travel from the nucleus to the ribosome, the viral protein Rev assisted by different host factors, seems to substitute the effects of splicing leading to the building of a viral ribonucleoprotein complex (RNP) that is efficiently exported from the nucleus and translated in the cytosolic compartment. The role of Rev and host DEAD-box RNA helicases eIF4AIII and DDX3 in the control of gene expression from the HIV-1 unspliced mRNA will be discussed.

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SYMPOSIUM 6
GREEN BIOLOGY: UNDERSTANDING MOLECULAR MECHANISMS IN PLANT SYSTEMS
Chair: Claudia Stange, Universidad de Chile

Bacteria that can modulate the plant life cycle: Beneficial interaction between *Arabidopsis thaliana* and the rhizobacteria *Burkholderia phytofirmans* PsJN

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Plants constantly interact with microbial communities inhabiting the rhizosphere and their internal tissues. Some of the mutualistic interactions involve Plant Growth Promoting Rhizobacteria (PGPR) that can increase plant growth and reduce biotic/abiotic stress susceptibility. We have approached these relevant biological interactions using the well-known PGPR *Burkholderia phytofirmans* PsJN and *Arabidopsis thaliana* plants. Thus, we studied the long-term effects of this strain in plants and the molecular mechanisms explaining its beneficial effects. Finding that a single inoculation period affects the whole life plant cycle, accelerating its growth rate, and shortening its vegetative period. Also, transcriptomic analyses showed that PsJN regulates the expression of several genes early in Arabidopsis development; some of them related with stress response and hormone pathways. Consequently, we have used genetic, chemical and transcriptional approaches to study the interplay of different plant hormones (such as auxin, ethylene and gibberellins) in the growth promotion induced by this PGPR. Interestingly, we have found that biosynthetic/signaling pathways related with these hormones may explain the diverse effects observed in the inoculated plants. Also, we have found that PsJN strain induces salt-stress tolerance in Arabidopsis under short and long-terms of stress exposure. PsJN primes plant salt-stress molecular responses involved in abscisic acid-dependent pathways; ion transporters; ROS scavenging; detoxifying and down-regulated genes related with jasmonic acid biosynthesis. Some of these changes are maintained over time. Overall, our results reflect a complex array of molecular and hormonal mechanisms underlying PGPR/plant interactions under favorable and adverse environmental growth conditions.

Involvement of the intracellular vesicle trafficking in the plant tolerance to salt stress

Ruiz-Lara, Simón[1]., Pérez-Díaz, Ricardo[1]., San Martín, Alex[1]., Soto, Flavia[1]., Madrid-Espinoza, José[1]., González, Claudia[2]., Pizarro, Lorena[3]., Norambuena, Lorena[3]., Blumwald, Eduardo[4]., [1]Instituto de Ciencias Biológicas Universidad De Talca. [2]Centro de Biología Molecular Vegetal, Facultad de Ciencias, Universidad de Chile. [3]Centro de Biología molecular Vegtal, Facultad de Ciencias, Universidad De Chile. [4]Department of Plant Sciences University of California, Davis. (Sponsored by Supported By Fondecyt 1140636.)

Adaptation of plants to abiotic stress may involve rearrangement of molecules from various cellular compartments. The transport of material to and from the specific compartments is carried out by an intracellular vesicle trafficking system,regulated by members of the large superfamily of small GTPases. Rab GTPases operate as a molecular switch that cycle between “active” and “inactive” states. This cycle is linked to the binding and hydrolysis of GTP, which is dependent upon the interaction with accessory proteins. Rab GTPases require: (i) guanine nucleotide exchange factors (GEFs) that facilitate GDP dissociation, (ii) GTPase activating proteins (GAP) that stimulate GTP hydrolysis and (iii) guanine dissociation inhibitors (GDI) that form soluble complexes with small GTPases. Recent evidence indicated that some Rabs and GDI isoforms are involved in the plant responses to abiotic stress. Here, we investigated the involvement ofthis molecular switch in the tolerance of tomato wild type *Solanum chilense* to salt stress. We identified a number of salt stress-induced genes, and studied their function(s) by their overexpression in tomato and Arabidopsis plants. Our results show that the overexpression of *SchRabGDI1* increased the endocytosis and the length of the roots. ShRabGDI1 and SchRabG3e proteins interact physically in the cytoplasm of the cell. The ShcRabGTPG3e identified would be responsible of driving the formation of pre-vacuolar structures that provide sites for the accumulation of excess cytoplasmic sodium prior to its storage into the vacuole. All transgenic plants analyzed showed an increased tolerance to salt stress.

Long distance K^+ transport in plants

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Potassium (K^+) is an important nutrient for plants and is recognized as a limiting factor for crop yield and quality. It serves as a cofactor of various enzymes and as the major inorganic solute is involved in maintaining plant cell turgor. K^+ also plays an important role in numerous metabolic processes, for example, it serves as an essential cofactor of enzymes. Interdisciplinary research in the past years bridging biophysics and plant physiology helped identifying additional roles of K^+ in plant growth. K^+ ions circulating in vascular tissues serve as a source of information on the growth status of the shoot and transmit it to the roots. Structure-function studies clarified the mechanism how specialized K^+ channels in xylem parenchyma cells read out this information and react flexibly with xylem K^+ loading on the shoot's demand for K^+. Additionally, by a systems biology approach combining diverse biophysical and plant physiology experimental approaches with computational cell simulation, it was shown that K^+ circulating in the phloem serves as a decentralized energy storage that can be used to overcome local energy limitations. Unique posttranslational modification of the Arabidopsis K^+ channel AKT2 taps this potassium battery, which then efficiently assists the plasma membrane H^+-ATPase in energizing the transmembrane phloem (re) loading processes under energy-limiting conditions.

“Integration of nitrogen gene networks and plant defense responses”

Vega, A[2]., Canessa, Paulo[1]., Hoppe, Gustavo[2]., Retamal, Ignacio[2]., Rubilar, Joselyn[2]., Moyano, Tomas[1]., Canales, Javier[3]., Gutiérrez, Rodrigo[1]., [1]Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica De Chile. [2]Departamento de Ciencias Vegetales, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica De Chile. [3]Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral De Chile. (Sponsored by Fondo Nacional De Desarrollo Científico Y Tecnológico FONDECYT-Chile 11110095 And 11140678, Millennium Nucleus Center For Plant Systems And Synthetic Biology (NC130030) And Millennium Nucleus For Fungal Integrative And Synthetic Biology (NC120043))

Nitrogen (N) is one of the main limiting nutrients for plant growth and crop yield. Despite its role as a nutrient, nitrate can also act as a signaling molecule that modulates gene expression of a wide range of plant processes. It is well documented that changes in nitrate availability, the main N source found in agricultural soils, influences a myriad of developmental programs and processes including plant defense responses. Plant N nutritional status influences their ability to respond effectively when challenged by different pathogens. However, the molecular mechanisms involved in N-modulation of plant susceptibility to pathogens are poorly characterized. In this work, we show that *Solanum lycopersicum* defense response to the necrotrophic fungus *Botrytis cinerea* is affected by plant N availability, with higher susceptibility in nitrate-limiting conditions. Genome-wide expression responses of tomato against infection by the fungus under contrasting nitrate conditions reveals that plant primary metabolism is affected by the fungal infection regardless of N regimes. This result suggests that differential susceptibility to pathogen attack under contrasting N conditions is not explained by a metabolic alteration. We used a systems biology approach to identify the transcriptional regulatory network implicated in plant response to the fungus infection under contrasting nitrate conditions. Interestingly, hub genes in this network are known key transcription factors involved in ethylene (ET) and jasmonic acid (JA). Our results provide insights into potential crosstalk mechanisms between necrotrophic defense response and N status in plants.

SYMPOSIUM 7
KEY ROLES OF AUTOPHAGY IN CHRONIC DISEASES

Chair: Eugenia Morselli, P. Universidad Católica de Chile
Alfredo Criollo, Universidad de Chile

Pathological Cardiac Remodeling: Role of the Unfolded Protein Response and Autophagy

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Cardiovascular disease (CAD) is the leading cause of mortality and mobility worldwide. Autophagy, a self-eating process, is involved in essentially every type of CAD. However, the role of autophagy remains largely elusive. To investigate the dynamic feature of autophagy in CAD, we created a transgenic mouse model expressing a tandem fluorescent protein GFP-RFP fused with LC3. Using this novel transgenic mouse, we analyzed the dynamic changes of autophagy in cardiac ischemia-reperfusion and doxorubicin-induced cardiotoxicity. We found the flux of autophagy is severely impaired by ischemia-reperfusion and doxorubicin treatment. Stimulation of autophagy flux strongly improves cardiac function and clinical outcomes. Mechanistically, we found the unfolded protein response is potently induced by various cardiac stresses, which may directly stimulate autophagy process. Our results show that the dynamic regulation of autophagy plays critical roles in cardiomyocyte homeostasis under stress and the unfolded protein response may serve as a direct upstream regulator of autophagy in heart, which may shed light on identification of novel therapeutic approaches to tackle heart disease.

Consumption Of High Fat Diets Rich In Saturated Fatty Acids Inhibits Hypothalamic Autophagy

Morselli, E[1]., [1]Physiology, Biological Sciences, Pontificia Universidad Católica De Chile.

Worldwide prevalence of obesity has more than doubled in the last decades and currently about 25% of the adult population is obese. This exponential rise in obesity has been associated with increased consumption of the so-called “Western-style” high fat diet (HFD), a type of diet high in saturated fatty acids (SatFAs), such as palmitic acid (PA). In mice, chronic consumption of HFD increases hypothalamic concentration of SatFAs and inhibits autophagy. *In vitro*, in N43/5 hypothalamic neuronal cells, exposure to pro-obesigenic concentrations of saturated fatty acid palmitic acid (PA) inhibits autophagic flux, suggesting that PA mediates the effects we are seeing *in vivo*. Interestingly, HFD consumption and/or gavage of pro-obesigenic concentrations of PA increase mRNA levels of *Gpr40,* a G-protein coupled fatty acid receptor activated by long chain fatty acids. Treatment with PA *in vitro* activates GPR40 causing intracellular Ca^{2+} release, an effect that is significantly inhibited by pretreatment with the GPR40 antagonist GW1100. Interestingly, in the same cell type, treatment with the GPR40 inductor, which promotes intracellular Ca^{2+} increase, inhibits autophagic flux. Consistently, treatment with the GPR40 inhibitor GW1100, which decreases intracellular Ca^{2+}, promotes autophagic flux, as shown by reduced p62 levels. These data suggest that GPR40 regulates autophagy by modulation of intracellular Ca^{2+}. Furthermore, these results show PA activates GPR40, thereby inhibiting autophagic flux.

Regulation of Autophagy by Polycystin-2

Criollo, Alfredo[1]., [1]Instituto de Investigacion en Ciencias Odontologicas, Facultad de Odontologia, Universidad De Chile. (Sponsored by FONDECYT Grant 1140908 And ACCDis FONDAP 15130011)

The mechanism of autophagy, the main cellular pathway in the turnover of proteins and degradation of organelles, is not totally understood. Mutations in the gene encoding for polycystin-2 (*pkd2*) are implicated in a number of human diseases, including autosomal dominant polycystic kidney disease (APKD). Key autophagy regulators such as mTORC1 and AMPK are dysregulated in APKD and treatment with rapamycin, a strong inductor of autophagy by chemical inhibition of mTORC1, is effective in the treatment of APKD patients, suggesting that mutations in pkd2 impair autophagy. In this study, we observe that down regulation of polycystin-2 with specific siRNAs inhibits starvation and rapamycin-induced autophagy in different cell types, such as neonatal cardiomyocytes, HeLa, U2OS and fibroblasts. Cardiomyocyte-specific polycystin-2 knock-out mice (PC2F/F αMHC-Cre mice) show impaired fasting and overload stress-induced autophagy in heart but not in other organs revealing a specific role for polycystin-2 in the regulation of autophagy in vivo. Polycystin-2 regulates autophagy through the mobilization of Ca^{2+}Cyt. Indeed Ca^{2+}Cyt is required to induce starvation or rapamycin-induced autophagy and depletion of PC2 inhibits autophagy by decreasing Ca^{2+}ER. Consistently, over expression of polycystin-2 induces autophagy by increasing Ca^{2+}Cyt, and this effect is polycystin-2 calcium channel activity dependent. In conclusion, polycystin-2 is a novel regulator of autophagy by controlling intracellular calcium homeostasis. This work was supported by FONDECYT grant 1140908 and ACCDis FONDAP 15130011.

Autophagy and plasma membrane domains.

Codogno, P[1]., [1]INSERM U1151-CNRS UMR 8253 Université Paris-Descartes/Paris V.

Macroutophagy (hereafter referred to as autophagy) is a tightly regulated intracellular degradation pathway essential for cellular homeostasis regulation, initiated by the formation of autophagosomes which engulf portions of the cytoplasm and organelles and fuse with the lysosome for material degradation. The origin of autophagosome is still unclear: multiple membrane sources, including the endoplasmic reticulum (ER), mitochondria, endosomes and the plasma membrane (PM) have been suggested to contribute to autophagosomal membrane biogenesis. In mammals, a platform for autophagosome biogenesis likely exists on the ER and recent studies suggest that mitochondrial membrane contact sites are the putative precise sites. In parallel, growing evidence suggests that ER-PM contact sites, principally linked to regulation of calcium homeostasis and lipid trafficking, may play more general physiologic function(s) via membrane dynamics regulation. In higher eukaryotes three ER-localized proteins, the extended synaptotagmins (E-Syt1, 2 and 3), play a crucial role in tethering the ER to the PM, notably via phosphoinositides local metabolism. Here we report the implication of ER-PM contact sites in the early stage of autophagosome biogenesis. In a second part of the talk we will discuss the role of the interplay between autophagy and the primary cilium, a microtubule-based structure that is continuous with the plasma membrane, in response to stress situations (starvation, shear stress). Overall, our results highlight the importance of the plasma membrane domains in the autophagosome biogenesis and in the regulation of the autophagic pathway.

SYMPOSIUM 8
WHEN BIOLOGY MEETS COMPUTERS: ONCODOMAINS, MICROBIOMES AND ALLOSTERIC NETWOKS

Chair: Daniel Almonacid, Universidad Andrés Bello
Cesar Ramírez-Sarmiento, Universidad de Chile

Sequence similarity networks: Phylogenomics tool for studying sequence relationships across large datasets

DE Almonacid[1,2], I Varas-Concha[1,2], M Alegría-Arcos[1], J Soto-Lambert[1], D Pizarro-Farías[1], J Bascur-Cifuentes[1], N Arriagada-Reveco[1], C Valdivia-Álvarez[1]., [1]Center for Bioinformatics and Integrative Biology (CBIB), Facultad de Ciencias Biológicas, Universidad Andrés Bello, Santiago, 8370146, Chile., [2]uBiome, Inc., San Francisco, CA 94103, United States

Next-generation sequencing is overflowing the public repositories with nucleotide sequences, and the predicted protein sequences they encode. This is an incredible time to work in the biological sciences, because the amount of novel data being discovered daily is unparalleled. Yet, to take advantage of the data deluge, tools to extract knowledge from large sequence datasets need to become commonplace in research laboratories.

Sequence similarity networks (SSNs) are a phylogenomics approach based on all-against-all pairwise comparisons of a group of sequences. They have a number of good properties, including they are fast to compute, robust to missing data, and allow easy identification of clades. Additionally, they correlate well with phylogenetic trees, and there is a variety of software that allows the generation of detailed images of networks. The only downside to SSNs is that just recently software to generate these networks has become available to the general public, so SSNs have not yet found widespread use.

Our groups have been using SSNs extensively during the past few years. First, I will describe methodological aspects of their generation. Then, I will present their central use in different research projects: i. Characterization of novel members of protein channels; ii. Search for enzymes with novel activities; iii. Defining oncodomains from cancer exomes; iv. Taxonomic characterization of 16S sequences from human microbiomes. Given that SSNs empower researchers to make use of large sequence datasets, we envisage they will become a widespread method in this new era of biology.
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Transcriptome editing promotes breast cancer progression through the regulation of cell cycle and DNA repair.

Sagredo, A Eduardo[2]., Sagredo, Alfredo I[2]., Blanco, Alejandro[2]., Morales, Fernanda[2]., Verdugo, Ricardo[2]., Olivier, Harismendy[1]., Marcelain, Katherine[2]., Armisen, Ricardo[3]., [1]Moores Cancer Center, Medical School, University of California San Diego. [2]Centro de Investigación y Tratamiento del Cáncer , Facultad de Medicina, Universidad De Chile. [3]Pfizer Chile, Center of Excellence in Precision Medicine

Among the most overexpressed genes in breast cancer, the presence of “adenosine deaminases acting on RNA” (ADAR) suggest the possibility that aberrant posttranscriptional modifications such as alternative splicing and RNA editing may lead to further tumor-specific transcriptome diversity adding a new level of complexity on top of known cancer genomic modifications at DNA level. In humans, the most common type of RNA editing is conversion of A to I, which is catalyzed by the double-stranded RNA (dsRNA)-specific ADAR family of proteins. Because of the miscellaneous effects of RNA editing on gene expression and function, it is possible that the misregulation of A-to-I RNA editing may play a role in tumorigenesis by either inactivating a tumor suppressor or activating genes that promote tumor progression. To test this possibility we used bioinformatic tools to generate a curated consensus list of genes edited by ADAR and a list of genes that are coexpressed with ADAR in breast cancer samples from TCGA patients. The intersection of these two lists of genes identifies 333 genes with gene ontologies heavily focused on “Cell Cycle” and “DNA repair” (Bonferroni corrected p value<10E-22 to 10E-5), and includes many genes directly involved in cancer. These correlations have a clinical meaning since TCGA breast cancer patients that overexpress ADAR have a significant decrease in their overall survival rate compared to patients with low or normal levels of ADAR1 expression in their tumors in a >10 years follow-up Kaplan Maier analysis.
Supported By FONDECYT 1151446 And CORFO 13CEE2-21602.

Your microbiome and citizen science, science at scale.

ZS Apte, S Rezvan-Behbahani, AJ Smith, I Varas-Concha, DE Almonacid, J Richman

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A healthy human adult carries more than 100 trillion microbial cells, which inhabit just about every part of our bodies. Studies have linked aspects of the microbiome to cardiovascular disease, obesity, mood and behavior, diabetes, numerous gut disorders, diet, metabolism and the immune system, to name a few. In fact, the role of the microbiome is so significant it has been referred to as the “forgotten organ”.

The institutions working on microbiome research are disconnected from the individuals whose health can be affected by their scientific discoveries. As a result, institutional agendas often overpower patient goals. Through a citizen science approach we allow everyone to explore their microbiome and discover how it is influenced by health and lifestyle, equipping people with the tools to create their own scientific studies.

Based on culture-independent high-throughput sequencing, and through a citizen science approach, we are interrogating at an unprecedented scale the microbes that inhabit our bodies. A large-scale self-sampling cohort study has the potential to reveal the depth and repeatability of correlations between microbiome composition and health conditions that could be translated into medical treatments.

In this talk, I will show some results on the analysis of taxa present in uBiome users data, the largest gut microbiome dataset in the world. I will then present some of the challenges faced in taxonomically annotating 16S sequences, including some illustrative examples. Finally, I will discuss some of the techniques we are using to categorize users based on the inferred taxonomy of their microbiome samples.

Allosteric Networks in Thrombin

Elizabeth A. Komives Department of Chemistry and Biochemistry, University of California San Diego, La Jolla, California, USA.

Serine proteases are found ubiquitously in both eukaryotes and prokaryotes. However, the dynamic motions of this largest peptidase family remain unknown. All serine proteases have a double β-barrel core surrounded by connecting loops and helices, but compared to the prototypical serine protease, chymotrypsin, thrombin has more extended loops that are thought to impart greater specificity. We analyzed apo-thrombin and active site-bound (PPACK-thrombin) using a combination of MD, accelerated MD and NMR. The ps-ns backbone dynamic motions were determined in both states using R_1, R_2, ^{15}N-{^1H}NOEs, and these were accurately recapitulated by conventional MD simulations. Longer timescale motions were captured in residual dipolar coupling measurements, which were used to calibrate AMD simulations. TROSY Hahn-Echo, and relaxation dispersion experiments revealed μs-ms motions in apo thrombin. The various surface loops move on different time scales as indicated by our inability to globally fit the relaxation dispersion curves to a single motional model. Community analysis of the AMD ensembles identified groups of residues linked through correlated motions and physical contacts. Some of these communities represented groups of residues for which the relaxation dispersion data could be globally fit to a two-state exchange model. These include the Na^+-binding loop and the 170s loop, both of which are involved in substrate recognition, and for which the motions were completely dampened upon PPACK-binding. Resonances from a third region, the N-terminus of the heavy chain and the γ-loop, also correponded to a “community” in the AMD simulations, and this region has been implicated in allostery invoked by thrombomodulin binding to exosite 1. The results suggest that apo-thrombin consists of a broad ensemble of states, which are conformationally selected by substrate and/or effector binding.

ORAL SESSION

Oral Sessions 1
MOLECULAR CELL BIOLOGY
Chair: Roxana Pincheira
Co-Chair: Clara Quiroga

Participation of cathepsin L in apoptosis of colorectal cancer cell lines subjected to metabolic stress

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Tumor cells that suffer from metabolic stress activate various signaling pathways to satisfy their bio-energy needs and survive. Recent research work has reported that both the lysosome as well as lisosomal proteases are involved in this process. In particular, cysteine proteases such as cathepsin L participate both in apoptosis as well as in autophagy processes, depending on the studied cellular line and the cellular stress. However, no studies with respect to its function during a metabolic type of stress have been performed. Thus, we studied the function of cathepsin L in the survival of colorectal cancer cells under metabolic stress. Our results showed a differential expression of nuclear and cytosolic cathepsin L in COLO320 and SW620 cell lines subjected to stress in a serum-free medium or in a medium without glucose. On the other hand, when the activity of cathepsin L was inhibited using a competitive inhibitor and subsequently cells were submitted to the aforementioned stress, a decrease in cellular survival were observed. Cellular survival was quantified by means of the MTT technique and by the levels of apoptosis, measured through detection of apoptotic markers, such as caspase-3 cleaved, and measurement of DNA degradation by means of an agarose gel and the comet assay. Results suggest that cathepsin L participates in a signaling pathway associated to the apoptosis process in colon cancer cells under metabolic stress.

RNP complexes regulating higher order structure of chromatin

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The modulation of the higher order structure of chromatin has profound implications in the regulation of nuclear processes, like transcription, replication, recombination or DNA repair. In those processes the DNA has to be accessible to allow the binding of regulatory proteins to switch the activity state of the genes. However, the dynamic processes involved and the mechanisms that change higher order structures of chromatin are still not clear. Recently we identified in Drosophila a novel mechanism of chromatin opening directed by an RNP complex that contain non-coding RNA and the decondensation factor 31 (DF31). In humans we identified the high mobility group nucleosome binding domain 5 (HMGN5) as a potential RNP candidate. This protein shows sequence similarities to DF31 and its localisation with euchromatin suggests a function in maintaining an open chromatin structure, as we have described for DF31. In this work we could show that HMGN5 has a specific RNA binding activity, and we are trying to identify its potential RNA interacting partners in the RNP complexes. To identify candidate targeting signals for the RNP mediated chromatin opening, we are integrating the analysis of the effect of HMGN5 in the global transcriptome profile, together with the mapping of the binding sites in the genome. Once having the candidate RNAs they will be tested in the chromatin compaction assay and also validated in vivo, in order to determine their role in the regulation of the chromatin architecture.

Characterization of thermodynamic and kinetic parameters of αvβ3 integrin and Syndecan-4 interaction with Thy-1 using a single molecule approach.

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We have shown that both αvβ3 integrin and Syndecan-4 are expressed on the surface of the astrocyte cell line DITNC1, and interact with neuronal Thy-1 to mediate bidirectional neuron-to-astrocyte communication. In astrocytes, these interactions promote cellular adhesion and migration, while in neurons, they inhibit neurite outgrowth. Our reported Surface Plasmon Resonance data show that Thy-1-Fc interacts directly with αvβ3-Fc through an integrin-binding site; however, accurate measurements of binding parameters were not possible due the αvβ3-Fc fusion protein employed was only available in the non-purified state as supernatants from transfected cells. Thy-1 also possesses a putative heparin-binding domain that is thought to mediate the interaction with Syndecan-4; nonetheless, direct binding of these molecules has not been demonstrated to date. Single-molecule studies constitute a direct approach to characterize protein-protein interactions. Thus, we used Optical Tweezers to quantify dissociation forces and determine thermodynamic and kinetic parameters of αvβ3/Thy-1 and Syndecan-4/Thy-1 interactions. To this end, Thy-1-Fc, αvβ3-Fc and Syndecan-4-Fc fusion proteins were attached to protein G-polystyrene beads. Laser beam-trapped beads coated with either αvβ3-Fc or Syndecan-4-Fc were allowed to contact the other bead coated with Thy-1-Fc, and after 5 seconds, beads were separated at a constant force loading rate (10 pN/s), until rupture of the interaction was observed. Rupture force histograms and Dudko-Hummer-Szabo equation were used to determine the off-rate at zero force (k_{off}^{0}), distance ($\Delta x^{\ddagger}$) and free energy ($\Delta G^{\ddagger}$) of the transition state. The results obtained provided evidence for a direct interaction between Syndecan-4 and Thy-1, with a k_{off}^{0} = 0.124 s^{-1}, $\Delta x^{\ddagger}$= 0.23 nm, $\Delta G^{\ddagger}$= 4.11 $k_B T$. In addition, αvβ3-Fc/Thy-1 interaction was also demonstrated. Because the αvβ3-Fc protein was only available in the non-purified state, we developed a mathematical strategy to filter non-specific interactions. This allowed us to estimate a k_{off}^{0} = $9.9e^{-3}$ s^{-1}, $\Delta x^{\ddagger}$= 0.42 nm, $\Delta G^{\ddagger}$= 7.76 $k_B T$ for αvβ3-Fc/Thy-1 binding. These results indicate that single-molecule force spectroscopy can be used to characterize bimolecular interactions, even when proteins are not available in the purified state. Furthermore, this approach yields important insights to protein-protein interactions involved in the communication between neurons and astrocytes.

Inhibition of autophagy and mitochondrial dysfunction in cancer cells under starvation conditions are linked to the tumor suppressor role of Caveolin-1

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Introduction: Caveolin-1 (CAV1) is a membrane protein that in the presence of E-cadherin functions as a tumor suppressor. Recently, in an *in vivo* model, we observed that CAV1, in the absence of E-cadherin, partially suppresses subcutaneous tumor formation by B16F10 mouse melanoma cells in C57BL6 mice. Autophagy is a highly conserved catabolic process that involves lysosomal degradation of cytoplasm components and serves as an adaptive response to different stress situations favoring mitochondrial metabolism and tumor growth. Here we determined whether tumor suppression by CAV1 is linked to inhibition of autophagy.

Methodology: We evaluated *in vitro* whether CAV1 modulated autophagy in B16F10 melanoma cells and MDA-MB-231 breast cancer cells under starvation conditions. Autophagic flux was determined under basal and starvation conditions for 5 hours in the presence or absence of chloroquine (CQ) by western blotting. Also, for B16F10 cells transfected with GFP-LC3 plasmid, the numbers of dots induced by starvation was determined using confocal microscopy. Oxygen consumption rates in basal and starvation conditions were determined using an oxygen electrode. In addition, we evaluated mitochondrial mass and distribution, mitochondrial membrane potential and intracellular ATP levels by confocal microscopy, flow cytometry and luminometry, respectively. Finally, *in vivo* tumor formation was evaluated in the absence or presence of chloroquine (60 mg/Kg). Expression of p62 protein in samples from tumors after 15 days was determined by western blotting.

Results: CAV1 decreased the autophagic flux both in basal and starvation conditions. Moreover, the inhibition of autophagy by CAV1 correlates with an increase in cell death induced by starvation. On the other hand, for CAV1-expressing cells, decreased oxygen consumption rates in both basal and starvation conditions without changes in mitochondrial mass or ATP levels were observed. *In vivo*, tumor suppression by CAV1 correlated with increased expression of p62 protein, suggesting a decrease in autophagy in these tumors. Finally, for mice inoculated with Mock cells and treated with CQ, a similar decrease in tumor volume was observed as for CAV1 cells.

Discussion: These results suggest that the inhibition of starvation-induced autophagy and promotion of mitochondrial dysfunction by CAV1 may contribute to its tumor suppressor role in the absence of E-cadherin.

Rheb promotes cancer cell survival through p27Kip1-dependent activation of autophagy.

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The small GTPase Rheb is the molecular link between TSC and mTORC1 complexes, both involved in a pathway that regulates key cellular processes. The imbalance of this pathway is frequently associated with proliferative disorders, including cancer. Thus, mTORC1 inhibitors have been used for tumor treatment, but without success because of tumor recurrence. This recurrence is likely due to the existence of mTORC1-independent mechanisms. Previously, we found that Rheb regulates the activity of AMPK and the intracellular localization of the cell-cycle inhibitor p27 in MEF Tsc2-/- and colon cancer cells, through an mTORC1-independent manner. However, the biological consequence of the Rheb-p27 axis in cancerous cells remained unclear. By using specific siRNAs, we found that Rheb is essential for survival of colon cancer cells under serum deprivation. Previously, it was reported that AMPK promotes autophagy through p27. Since autophagy can be pro-tumorigenic, we evaluated whether the Rheb-p27 axis was involved in cell survival through autophagy. Our studies demonstrated that Rheb promotes the survival of cancer cells through p27-induced autophagy. In addition, our results indicate that the role of p27 in autophagy stands on its amino-terminal portion, where the CDK-inhibitory domain is located. Finally, by using an *in vivo* model, we confirmed that Rheb is involved in the tumorigenic capacity of colon cancer cells. The results of this work highlight the relevance of the Rheb-p27 axis in mTORC1-independent tumorigenic processes, still relevant for designing therapies for patients with tumor recurrence after treatment with mTORC1 inhibitors.

Sall2 is required for pro-apoptotic Noxa expression and genotoxic stress-induced apoptosis by doxorubicin.

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The Sall2 transcription factor is deregulated in several cancers; however, little is known about its cellular functions, including its target genes. We investigated the role of Sall2 in the context of cellular response to genotoxic stress in primary mouse embryo fibroblasts (MEFs). We found that the levels of Sall2 mRNA and protein are dynamically modulated in response to doxorubicin. At early times of stress Sall2 is downregulated, but increases under extension of the stress. Based on caspase-3/7 activities, expression of cleaved poly (ADP-ribose) polymerase, expression of cleaved caspase-3 and induction of proapoptotic proteins, Sall2 expression was correlated with cellular apoptosis. Consequently, *Sall2*$^{-/-}$ MEFs have decreased apoptosis, which relates with increased cell viability in response to doxorubicin. Searching for putative Sall2 targets that could mediate its role in apoptosis, we identified proapoptotic NOXA/PMAIP1 (phorbol-12-myristate-13-acetate-induced protein-1). We demonstrated that Sall2 positively regulates *NOXA* promoter activity. Conserved putative Sall2-binding sites at the Noxa promoter were validated *in vitro* by EMSA and *in vivo* by ChIP experiments, identifying *NOXA* as a novel Sall2 target. In agreement, induction of Noxa protein and mRNA in response to doxorubicin was significantly decreased in *Sall2*$^{-/-}$ MEFs. Studies in Jurkat cells support the existence of the Sall2/Noxa axis, and the significance of this axis on the apoptotic response to doxorubicin in cancer cells. Our study highlights the relevance of Sall2 in the apoptotic response to extended genotoxic stress, which is important for understanding its role in normal physiology and disease.

Oral Sessions 2
BIOMEDICINA-SIGNALING
Chair: Veronica Burzio
Co-Chair: Rody San Martin

IMPAIRED INSULIN REGULATION OF ADENOSINE TRANSPORT OCCURS IN DIABETIC GLOMERULI.

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Introduction. Insulin resistance impacts on renal cell physiology contributing to diabetic nephropathy (DN). Progression of DN has been linked to increased adenosine levels and in turn to pathogenic signaling through adenosine receptors. Extracellular adenosine availability is controlled by the uptake activity mediated by the equilibrative nucleosides transporters ENT1 and ENT2. Lower uptake activity in diabetic glomeruli was recognized. Thus, the aim of this study was to recognize signaling alterations affecting ENT1 and ENT2.

Methodology. Adenosine uptake (2,3-[^{3}H]adenosine, 60s, 22°C) was assayed in purified glomeruli from healthy and streptozotocin-induced diabetic rats. ENT1-mediated transport is sensitive to 1µM NBTI, whereas ENT2 is sensitive to 2mM hypoxanthine. Glomeruli were preincubated with 5mM or 25mM D-glucose for 24h and exposed to 10nM insulin, 1µM GF109203X, 50µM PD98059 and 10µM H89 for 8 h. The adenosine amount was quantified by HPLC.

Results. In isolted glomeruli from healthy rats, high D-glucose concentration decreases the activity of ENT1 and ENT2 involving PKC and MAPK signaling molecules. Insulin reversed this effect by restoring the activity of these transporters involving probably PKA activation. The levels of extracellular adenosine were increased when the ENTs uptake activity was reduced and restored by insulin. In contrast, the transport activities in isolated glomeruli from diabetic rats were decreased, and insulin was unable to restore adenosine uptake. Consequently, levels of adenosine were found to increase in diabetic glomeruli.

Conclusions. Lack of insulin response impedes rescue of adenosine transport activity mediated by ENT1 and ENT2, thus supporting the chronically augmented adenosine in diabetic glomeruli.

Similar Rho kinase activation in circulating leukocytes and cardiovascular tissue in rats with high angiotensin converting enzyme levels

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The Rho/Rho kinase (ROCK) signaling pathway is responsible for cellular proliferation, migration, differentiation and gene expression which contributes to vascular tone and remodeling. ROCK activity is usually measured by determining phosphorylated levels of its downstream protein myosin phosphatase target subunit-1 (p-MYPT1). Another ROCK target is the ezrin, radixin and moesin (ERM) family protein complex, which also facilitates remodeling. ROCK activity in circulating leukocytes is increased in humans with hypertension, heart failure and metabolic syndrome. On the other side, high levels of plasma ACE induce ROCK activation in the aortic wall in the rat (Rivera, 2007). However, the relationship between ROCK activation in leukocytes and cardiovascular tissue is unknown. Accordingly, we determined p-MYPT1 and ERM phosphorylation (p-ERM) levels by Western blot simultaneously in circulating leukocytes, aortic wall and left ventricle (LV) in rats with genetically determined high and low ACE levels (homozygous F2 Brown Norway, BN, and Lewis, L, respectively). Results: compared to L rats, p-MYPT1 was increased in BN rats by 69, 79 and 61 % in leukocytes, aorta and LV, respectively, $p< 0.05$). A significant linear correlation was observed between p-MYPT1 between leukocytes and aortic and LV ($r = 0.497$ and 0.458, respectively). p-ERM levels were increased by 51, 92 and 85 % in leukocytes, aorta and LV, respectively ($p< 0.05$) and a significant correlation was observed between leukocytes and aortic and LV ($r = 0.46$ and $r= 0.702$, respectively). In conclusion, non invasive ROCK activation in circulating leukocytes robustly reflects cardiovascular ROCK activity. Fondecyt 1121060 (JEJ, MPO), FONDAP 15130011 (MPO).

The Sall2 tumor suppressor protein is regulated by Casein Kinase 2

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Sall2 is a transcription factor with critical roles during development, including neurogenesis and eye formation. Importantly, Sall2 has also been associated with cancer, although its contribution to the disease remains controversial. Sall2 is down-regulated in several tumor types and its activity promotes cell cycle arrest and cell death. Despite the physiological and pathophysiological roles of Sall2, little is known about its regulation. By using bioinformatic tools we identified several putative phosphorylation sites in Sall2 protein sequence. These sites are located within a potential and highly conserved PEST motif and match the consensus sequence for Casein Kinase 2 (CK2)-mediated phosphorylation. Here, we demonstrated that Sall2 is phosphorylated and regulated by Casein Kinase 2 (CK2), a ubiquitous and constitutively active kinase whose activity is increased in cancer cells. CK2 promotes both cell survival and proliferation by regulating stability, activity and/or subcellular localization of its target proteins, including tumor suppressors. To determine whether Sall2 is regulated by CK2, we used several approaches including pharmacological inhibition of CK2, mass spectrometry analysis, immunofluorescence, immunoprecipitation, *in vitro* kinase assays and site directed mutagenesis. Our results indicate that Sall2 is a CK2 target *in vitro*. In cells, CK2 interacts with Sall2 and triggers the ubiquitination and subsequent degradation of Sall2 in a proteasome-mediated fashion. Moreover, phosphorylation of Sall2 at S763 and T778 was greatly reduced under CK2 inhibition, suggesting that these are the most important CK2-dependent phospho-sites *in vivo*. In conclusion, we have identified Sall2 as a new CK2 target. Our results support a novel regulatory mechanism for Sall2 expression and function, providing new evidence for the role of both proteins in cell survival and proliferation, two deregulated processes in cancer.

ADENOSINE RECEPTORS CONTROL THE MIGRATION AND INVASION ABILITIES OF GLIOBLASTOMA MULTIFORME CELLS

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Introduction: The most aggressive type of brain tumor is Glioblastoma Multiforme (GBM). An important limiting factor for its treatment is the invasiveness ability of this neoplasia that allows infiltrate into the healthy tissue. Recent studies have shown that the presence of a subpopulation called Glioblastoma Stem-like Cells (GSCs) are responsible for the invasive capacity. On the other hand has been reported that the activation of adenosine receptors (ARs) would promote epithelial to mesenchymal transition (EMT) favoring migration/invasion processes in cancer. This work aimed to evaluate the effect of the blockage of adenosine receptors on the migration/invasion abilities in GBM. **Methods:** To evaluate the migration of cells, wound healing and Extracellular Matrix (ECM) assays were performed. For migration/invasion, the agarose spot and ECM-coated transwell assays were made. For evaluate effects of ARs, cells were treatment with selective antagonists: A_1 (DPCPX), A_{2A} (ZM241385), A_{2B} (MRS1754) and A_3 (MRS1220) ARs. A_3AR Knockout of A_3AR was generated ($U87^{KO}$) by CRISPR/Cas9 system. Finally the effect of MRS1220 on EMT was tested *in vivo*. **Results:** The assays showing a decrease migration/invasion abilities of adherent cells and GSCs treated with MRS1754 and MRS1220. The expression of EMT markers decrease with MRS1754 but more strongly with MRS1220. Similar effect were observed in $U87^{KO}$ cells. *In vivo* assays demonstrated that MRS1220 were able to mediate regression of tumors and immunohistochemically analysis showed decreased expression of EMT markers. **Conclusions:** The migration/invasion capacities of GBM cells decrease through blockade of A_{2B} and A_3 ARs, representing a new therapeutic alternative for GBM.

Role of TNF-α and oxLDL/LOX-1 pathway on cardiac myofibroblast phenotype

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Introduction: TNF-α and oxidized LDL (oxLDL) levels are elevated in chronic inflammation and it leads to development of cardiac fibrosis. Cardiac myofibroblast play a key role in fibrosis, however the effect of TNF-α and oxLDL on CMF phenotype is not known. **Objective**: To study the effect of oxLDL and TNF-α on the morphology CMF *in vitro*. **Methods:** Adult rat CMF culture was made. CMF were stimulated with TNF-α and oxLDL, then, we evaluated levels of α-SMA (differentiation marker), LOX-1 (oxLDL receptor) and NADPH oxidase (NOX; downstream element of oxLDL/LOX-1 pathway) by Western blot. CMF morphology was observed by immunofluorescence in response to stimuli. Also, we used siRNA to silence LOX-1 and NOX and we evaluated levels of α-SMA. **Results:** TNF-α induced a phenotypic disruption and decrease in LOX-1 and α-SMA levels in CMF.oxLDL induced activation of NOX and increasing the level of its own receptor (LOX1). Both processes were inhibited by TNF-α.oxLDL attenuates the phenotypic disruption induced byTNF-α and LOX-1 silencing inhibited α-SMA increase of CMF. **Conclusions:** oxLDL/LOX-1 pathway may play a key role on CMF phenotype, suggesting it as a novel pharmacological target on the treatment of cardiac fibrosis.

Triterpenic saponins from Quillaja extract: new therapeutic molecules to battle the cancer.

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Gastric cancer (GC) has generated interest of researchers due to increased cases per year and poor treatments. This is the second leading cause of death from cancers worldwide and the leading cause in Chile. Based on scientific background, which show that saponins are effective as anticancer agents, we evaluated the effects of these compounds, on gastric cancer tumoral cells. In Chile, a prominent source of saponin is *Quillaja saponaria*. The aim of this study was to investigate the mechanisms of action of saponins extracts from *Quillaja saponaria* on GC cells SNU-1 and KATO III. The cytotoxic effect of the extracts (QD, FS2, AQ) was evaluated by MTS and LDH assay. Mechanism of cell death and mitochondrial function was determined using, Rho/PI/AV by flow cytometry and caspases activation. All fractions under study had cytotoxic effect on cell lines. QD exhibited at effect stronger than other fractions. We evaluated the effect of different extracts on the integrity of cell membrane by LDH. At concentrations higher than 50 µM, all fractions increased the permeability of cell membrane. Moreover, FS2, QD and AQ triggered the apoptotic cascade and lowered the viability cells; suggesting the pass of saponins to cytoplasm to activate the caspase mechanism leading to cell death. Previous results showed that these molecules not produce these effects in normal cell lines. Our results demonstrate the potential therapeutic of these natural extracts from the Quillaja tree. It would be interesting to try lower doses to assess other effects described in literature, such as antioxidant or anti-inflammatory effect.

ORAL SESSIONS 3
COMPUTATIONAL BIOLOGY AND INFORMATICS
Chair: José Martinez
Co-Chair: Julio Caballero

The dimerization interface of paramyxovirus matrix proteins is structurally and evolutionary conserved.

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Paramyxoviruses are enveloped, single-stranded, negative-sense RNA viruses of the family *Paramyxoviridae*, order *Mononegavirales*. Human respiratory syncytial virus (hRSV), a paramyxovirus, is the major cause of bronchiolitis and pneumonia in children. The viral matrix (M) protein associates with membranes and plays a central role in viral assembly and budding. Paramyxovirus M proteins are dimers and form a characteristic square-shaped quaternary structure. Oligomerization of M was shown to be critical to hRSV infectious virus production and the protein is discussed as a potential drug target. We have characterized the dimerization interface of paramyxovirus and other mononegavirus M proteins and identified important anchor residues by estimating the relative buried surface area of interface residues. Important interface residues were on average more conserved than other surface residues. Based on these analyses we have generated a structural model of an alternative hRSV-M dimer conformation. Confirmation of this model via dimerization interface mutants and size exclusion chromatography indicates that arginine 99 participates in the dimerization interface in a conformation different from two recent crystal structures. Arginine 99 is in fact solvent exposed in the published crystal structures while it is >99% buried in our model. This suggests that molecular modeling augmented by structural and evolutionary analysis may complement our structural understanding of hRSV-M dimerization.

Comparative bioinformatic study of the transcriptional regulation of the receptor for oxidized low-density lipoprotein LOX-1 in rat, mouse and human and its potential role in heart diseases.

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Introduction: LOX-1 receptor oxidized low density lipoprotein (oxLDL), is considered a risk factor for cardiovascular disease (CVD) as atherosclerosis and heart failure. The comparative bioinformatic study of the transcriptional regulation of gene encoding LOX1 (OLR1) allows choosing models study and potential therapeutic targets in coronary diseases. **Objective**: The objective of this work is to identify potential sequences for expression regulation of OLR1 which is associated with CVD. **Methodology**: 1) A region of 1000 bp was taken as the gene promoter and sequences for transcription factor binding (TFBSs) were scanned using Lasagna 2.0 software. 2) Using DNAFSMiner software the alternative polyadenylation sites (PAS) was revealed on the transcript corresponding to the entire gene. **Results**: 1) Putative TFBS for AP-1, BRCA1, NKX1-3 and BACH1 were found on the OLR1 gene promoters of human, mouse and rat. 2) We detected PAS located distal and proximal to the 5'-UTR in human OLR1 transcript. **Conclusions**: Transcriptional regulation of LOX-1 is tissue and cell specific. Phenomena involving alternative polyadenylation, transcripts interference according to the genetic context of gene OLR1, and potential transcription factors in cardiac diseases are AP-1, by way of non-canonical TGF-β1; BRCA1, by canonical pathway of TGF-β1 activating Smad3; NKX1-3, joining co-factor MRTF 2 among others. These phenomena could acting coercively be responsible for isoforms and increased expression of LOX1 in cardiac pathologies.

Impact of the threading process on the folding mechanism determined by mechan**ical untie of a knotted protein.**

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Proteins containing knots must thread an extreme of its polypeptide backbone in order to reach the native structure. This process must occur the first time that the protein folds. However, once the knot has been established in the native state, the structure could unfold spontaneously accordingly to their own stability. Therefore, knotted proteins have an additional folding route if folding is initiated when the polypeptide chain remains knotted in the unfolded state. In this work we manipulated the topology of the unfolded state of a trefoil knotted protein, MJ0366 from *Methanocaldococcus jannaschi*, to compare its folding mechanism starting from the unknotted-unfolded state or from the knotted-unfolded state.

We mechanically unfold MJ0366 using optical tweezers. Therefore, two mutants were created, one to tight the knot and other one to untie it. When the knot was tightened, MJ0366 showed a simple two state folding mechanism characterized by single unfolding and refolding transitions. The contour length calculated from the unique transition was around 21 nm. This value is about 9 nm shorter than expected for the full extended polypeptide chain (30 nm), which could be explained by the presence of a tight knot. Conversely, in the case of the MJ0366 mutant, whose knot is untied upon pulling, were observed multiple unfolding-refolding transitions. These results suggest that threading of the polypeptide chain creates a rougher energetic landscape for folding, but once the knot is formed, the folding landscape becomes smooth.

Folding topology determines substrate binding order in the ribokinase superfamily

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Ribokinase superfamily comprises ADP-dependent and ATP-dependent enzymes where the nucleotide binding site is located in a β-meander module at the C-terminal region. Interestingly, only the ATP-dependent enzymes have a true β-meander module, whereas ADP-dependent enzymes have a non-circular permutation (NCP) in this region. Given that the permutation is the major structural difference between ADP and ATP-dependent kinases, it could be responsible for the nucleotide specificity. We introduce, by permutation, an ATP-dependent topology in the homologous ADP-dependent glucokinase from *T. litoralis* (perGK-ADP). Size exclusion chromatography and circular dichroism spectra show that both wild type and permutated enzyme eluted as monomers with similar hydrodynamic behavior, and the same secondary structure content. Kinetic assays demonstrate that even in presence of 10 mM ATP, perGK-ADP has not activity, without any significant change in the use of ADP. To test if the NCP has an impact in the kinetic constants and substrate binding order we determine the kinetic mechanism through classical protocols. The results demonstrate that perGK-ADP presents an ATP-dependent substrate binding order being the reverse of the order observed in the ADP-dependent wild type enzyme. Also perGK-ADP apo, enzyme-glucose and enzyme-glucose-ADPβS structures were determined by X-ray crystallography, which show ligand-induced conformational changes. The results demonstrated that although the permutation has no effect on nucleotide preference, it provokes a change in substrate binding order that correlate well with those observed in the crystal structures. Also, they demonstrated that during the evolutionary history of ribokinase superfamily folding topology dictates the substrate binding order.

The distance between exons and their nearest repeat plays a role in Repeat Fusion Transcript (RFT) formation in mouse genome.

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Chimeric or fusion transcripts between repeat elements and genes are common in cancer cells, but many examples have also been described in wild type cells. The mechanisms by which Repeat Fusion Transcripts (RFTs) are formed are speculative. Due to the large number of repeats in genomes their bioinformatic prediction presents a challenge. Previous work has used TopHat and Bowtie to find chimeric candidates in mouse genome. The predictions are a set of 466 RFTs, from which 17 were confirmed experimentally through PCR (10% of false positives). The predictions are uniquely mapped in the mouse genome (mm9). In this work, we analyzed the set of predicted RFTs to generate hypothesis of mechanisms of RFT formation. In the set, at least two mechanisms were recognized. In the main mechanism, 95% of sample fusions happen between exon and repeat in the same strand. In the second mechanism, 5% of sample fusions happen between repeats and exons in different chromosomes or in the same chromosome but different strands. 63% of fusions happen between the exon and the opposite strand of the annotated repeat. Finally, we tested the hypothesis that RFTs are formed by nearest repeat sequence from the exon to which it is fused. To that end, several statistical distributions of distances between exons and their nearest repeats were calculated bioinformatically using the mouse genome (mm9), repeat predictions from RepeatMasker and the gene annotation of mm9. The p-values calculated through exact tests do not reject the hypothesis of fusion of exons to nearest repeat.

Interdomain contacts and RNA polymerase control native state interconversion of the transformer protein RfaH on a dual-funneled landscape.

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RfaH is a virulence factor from *Escherichia coli* commonly referred as the first example of a **transformer protein**. Its C-terminal domain (CTD) undergoes a dramatic α-to-β conformational rearrangement, changing both its structure and function. The CTD in its α-helical fold is stabilized by interactions with the N-terminal domain (NTD), masking an RNA polymerase (RNAp) binding site until a specific recruitment site is encountered. Domain dissociation is triggered upon binding to a specific DNA element in the transcription elongation complex, allowing the NTD to interact with RNAp to facilitate transcription while the CTD refolds into a β-barrel that interacts with the ribosome to activate translation. Since this phenomenon is experimentally challenging, their structural details remain to be elucidated. Here, we explore the mechanism of the conformational switching of RfaH in the full-length protein using a dual-basin structure-based model. Our simulations capture several features described experimentally, such as the requirement of disruption of interdomain contacts to trigger the α-to-β transformation, confirms the roles of previously indicated residues E48 and R138, and suggests a new important role for F130, in the stability of the interdomain interaction. These native basins are connected through an intermediate state that builds up upon binding to the NTD and shares features from both folds. We also examine the competitive binding between RfaH-CTD and RNAp for the NTD, showing that RNAp binding favors the β fold. Our study shows that native-biased models are appropriate for interrogating detailed mechanisms of the structural rearrangements during the native state interconversion of RfaH.

ORAL SESSIONS 4
GENE EXPRESSION
Chair: Ariel Castro
Co-Chair: Julio Tapia

The HIV-1 Rev protein substitutes the effects of splicing on nuclear export and translation to promote efficient Gag synthesis from the unspliced mRNA

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Human Immunodeficiency Virus type-1 (HIV-1) gene expression involves the synthesis of a complex transcriptome including a subset of completely and incompletely spliced transcripts and one unspliced transcript. As cellular mRNAs, completely spliced transcripts follow the classical gene expression pathway in which splicing stimulates the rates of nuclear export and translation through the recruitment of different adaptor proteins such as SR proteins and the exon junction complex (EJC). In sharp contrast, the HIV-1 unspliced mRNA, which codes for the major structural protein (Gag), is retained and degraded in the cell nucleus unless the viral protein Rev is present. Here, we show that recruitment of Rev to the unspliced mRNA is sufficient to substitute the effects of splicing on nuclear export and translation. Interestingly, we observed that Rev drives the recruitment of the EJC core component eIF4AIII to the unspliced mRNA to promote Gag synthesis. These data reveal a novel mechanism by which a viral protein interconnects nuclear export and translation of an unspliced mRNA reminiscent to the effects of splicing on cellular transcripts.

The HBZ protein in the human T-cell lymphotropic virus type 1 (HTLV-1) is regulated at translational level by differents translation initiation mechanism.

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During its replication, HTLV-1 synthesizes two antisense messenger RNAs (mRNAs) from the provirus 3'LTR that encode for different versions of the HBZ protein. HBZ is fundamental for the viral replicative cycle and is associated with the pathogenicity caused by HTLV-1. The SpHBZ mRNA corresponds to a splice version while the UsHBZ mRNA is an unspliced mRNA. Both mRNAs are different only in their 5' untranslated regions (5'UTR) and are equally expressed in cells recovered from infected patients. Strikingly, the SpHBZ protein is more abundant than the UsHBZ protein, suggesting a possible regulation at the level of mRNA translation initiation. In this work, monocistronic vectors harboring the 5'UTR of SpHBZ or UsHBZ mRNAs fused to the sequence of the firefly luciferase gene (FLuc) were used to evaluate a possible role of the 5'UTR in the regulation of HBZ expression. Results show that the SpHBZ mRNAs translated better than the UsHBZ mRNA version in both the rabbit reticulocyte lysate (RRL) *in vitro* system and in cells. When the possibility of an IRES element within the 5'UTR of SpHBZ or UsHBZ is evaluated, results show that only the 5'UTR SpHBZ harbors IRES activity. Together these result shows that the expression of the HBZ protein is regulated at the level of translation. Furthermore, data show that translation initiation from the SpHBZ mRNA can occur via an IRES element while translation initiation driven by the 5'UTR of the UsHBZ mRNA is exclusively cap-dependent.

Decoupling circadian clock protein turnover from circadian period determination

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Circadian clocks are endogenous molecular timekeepers, conferring daily rhythms to a large number of biological processes within the cell. These clocks are present in various organisms and have appeared in different evolutionary lineages, impinging close to 24 hours rhythms in the regulation of gene expression, physiology and behavior. This coordinated rhythmicity, therefore, provides organisms with the ability to anticipate predictable cyclic environmental fluctuations. The mechanistic basis of eukaryotic circadian oscillators in model systems as diverse as Neurospora, Drosophila, and mammalian cells is thought to be a transcription-and translation– based negative feedback loop, wherein progressive and controlled phosphorylation of one or more negative elements ultimately elicits their own proteasome mediated degradation, thereby releasing negative feedback and determining circadian period length. The *Neurospora crassa* circadian negative element FREQUENCY (FRQ) exemplifies such proteins; it is progressively phosphorylated at more than 100 sites, and strains bearing alleles of *frq* with anomalous phosphorylation display abnormal stability of FRQ that is well correlated with altered periods or apparent arrhythmicity. Unexpectedly, we unveiled normal circadian oscillations that reflect the allelic state of *frq* but that persist in the absence of typical degradation of FRQ. This manifest uncoupling of negative element turnover from circadian period length determination is not consistent with the consensus eukaryotic circadian model

Identification of Sall2 transcriptional targets in response to genotoxic stress

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Cellular stress response is a universal mechanism for adaptation and survival of the cell against several insults, including temperature changes, mechanical stress or toxin exposure. In this context, cells that are unable to adapt and overcome the stress will die. The induction of genotoxic stress by chemotherapy is one of the first line treatments to eliminate neoplastic cells through apoptosis or programmed cell death. Our laboratory has characterized the Sall2 transcription factor, conserved among vertebrates, as a stress-response molecule, and demonstrated both *in vitro* and *in vivo* its requirement in the cell death-response caused by genotoxic stress. Few transcriptional targets of Sall2 are known, thus, additional targets most likely remain to be identified. We isolated mouse embryonic fibroblasts (MEFs) from a murine model deficient in Sall2 and from its normal counterpart, and use them to characterize the Sall2-dependent transcriptional response upon genotoxic stress. We isolated RNA from these cells and we performed and RNA-seq experiment. The analysis of the RNA seq data started off with the alignment of the reads with a splice–aware aligner such as Tophat. The quantification of genes/transcripts was done using HTSeq code and a count-table was generated. We applied a recent published strategy “Remove Unwanted Variation from RNA-Seq Data” or RUVSeq in order to normalize the gene-counts, and then we used the bioconductor package edgeRun to address differential expression of genes. We also assessed differential expression of transcripts and performed gene ontology analysis with DAVID database. Interestingly, we found by GO classification a cohort of genes involved with the immune system and inflammation, cell adhesion and response to wounding both in controls and treatments situations. Also we notice that the differential expression solely in transcripts between Sall2 wild type and null increases 10 fold with the treatment with doxorubicin, and these transcripts came from immune, developmental and cell-cycle related genes. Finally, we performed further validation of the analysis using qPCR. With this experimental outline, we are expecting to gain insights of how Sall2 contributes to the transcriptional program under normal and genotoxic stress-induced conditions.

HIV-1-mediated endolysosome translocation: Impact on viral RNA localization and *host metabolism*

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Mammalian target of rapamycin (mTOR) kinase couples metabolic and stress signals to pathways that mediate cellular growth and proliferation. mTOR maintains the activation of downstream effectors important for host cell metabolism including mRNA translation, cell proliferation and catabolic processes such as autophagy. mTOR activation requires late endosome/lysosome recruitment via Rag GTPases. In earlier work, we found that RagA co-precipitated with the HIV-1 RNP and in situ analyses revealed co-localization with mTOR in cytoplasmic punctae. Using confocal microscopy, we assessed the HIV-1-mediated efects of HIV-1 on mTOR. Oxidative stress mediated the predominant accumulation of mTOR to the perinuclear region, colocalizing with *lysosomal-associated membrane protein* 1 (LAMP1), but not with TIAR. In striking contrast, HIV-1 induced the peripheral distribution of LAMP1/mTOR and strikingly, RagA depletion impaired this. Phospho-S6K-1 and -4E-BP1 were induced to the same extent by oxidative stress in either mock or HIV-1-expressing cells. Finally, HIV-1 did not reverse mTOR inhibition in cells treated with mTOR inhibitors. These findings demonstrate that HIV-1 modulates mTOR activity and localization by two distinct mechanisms: one that targets an event upstream of mTOR and another that subverts viral trafficking, which in turn, seems to be dependent on the demonstrated interaction between HIV-1 and Rag-GTPase.

Differential response of three putative Wnt/b-catenin target genes, *cx43*, *c-myc* and *dax1* in 42GPA9 (mouse adult Sertoli) cell line.

LOPEZ, C[1]., Cereceda, K[1]., Montecino, M[2]., Meisterernst, M[3]., Slebe, JC[1]., Concha, II[1]., [1]Bioquimica y Microbiología, Ciencias, Universidad Austral De Chile. [2]Centro de Investigaciones Biomédicas Universidad Andrés Bello. [3]Institute for Molecular Tumor Biology University Muenster. (Sponsored by FONDECYT 1110508 (IC), 1141033 (JCS). CL: CONICYT And MECESUP AUS 1203 Fellowship.)

Sertoli cells are the nutritional and metabolic support of germ cells. Wnt/β-catenin signaling is important for the development of the seminiferous epithelium during embryonic age, however after birth this pathway is downregulated. Transgenic mice where β-catenin is constantly activated have altered spermatogenesis. Cx43, c-Myc and Dax1 are important proteins for testicular development during embryonic age. The three genes have WRE within their promoters and in transgenic mouse models, c-Myc and Dax1 are upregulated possibly affecting Sertoli cell functionality.

Here, we evaluated whether LiCl or Wnt3a treatments induce upregulation of *cx43*, *c-myc* and *dax1* geneexpression in 42GPA9 cells, and the possible molecular mechanism involved in the differential response of these genes.

Nuclear translocation of b-catenin was evaluated by western blot. mRNA abundance was determined by RT-qPCR and histone marks and b-catenin promoter occupancy at the WRE was assessed by ChIP analysis.

Sertoli cells responded to treatments, accumulating b-catenin within the nucleus, activating *axin2* transcription. In mES cells *cx43* and *c-myc* genes were upregulated under LiCl treatment but not *dax1*. Stimulated 42GPA9 showed a 2-fold increase of *cx43* mRNA, while *c-myc* and *dax1* mRNA were not affected. Histone marks of activation such as H3K9Ac and H3K4me3 were found only in cx43 and c-myc WRE although b-catenin was recruited in cx43 and dax1 WRE.

These findings suggest that *c-myc* gene is not a direct target of b-catenin upon activation of the Wnt/b-catenin signaling pathway compared to *cx43* gene in 42GPA9 cells.

ORAL SESSIONS 5
PROTEIN STRUCTURE-FUNTION AND MICROBIOLOGY
Chair: Ariel Castro
Co-Chair: Julio Tapia

Theoretical study of the iron entry route into *Pyrococcus furiosus* ferritin.

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Ferritin is a large protein cages that control the reversible formation of iron-oxy biominerals in a cavity with a diameter of 6-8 nm. Access to the inner cavity of ferritin is available through channels with 3- and 4-fold symmetry. It is well known that the 3-fold channel is the main iron entry route in mammalian ferritin. However, the 3-fold channel in prokaryotic ferritin is in general hydrophobic with the exception of *Pyrococcus furiosus* ferritin (PfFtn). Therefore, we study the properties of the 3- and 4-fold channel of PfFtn by molecular dynamics (MD). The same analysis was performed in Human L-chain ferritin (L-HuFtn) in order to compare the structural and electrostatic properties of these two proteins. In order to determine the propensity of cations to penetrate ferritin, we calculated the radial distribution function of sodium ions as a function of the center of mass of each protein. Our results show a high density of sodium ions inside the 3-fold channel of L-HuFtn, in agreement with its role as iron entry route. However, density of sodium ions is lower for the 3-fold channel of PfFtn, despite to present a similar electrostatic potential as the L-HuFtn 3-fold channel. Instead, the PfFtn show a high density of sodium ions in its B-channel, this channel has been reported as the iron entry route of bacterioferritin, another member of this protein family. Our results suggest that the B-channel could be the main iron entry route in PfFtn and probably in bacterial ferritins.

Polypyrimidine tract-binding protein binds to the 5'untranslated region of the mouse mammary tumor virus mRNA and stimulates IRES-mediated translation initiation.

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The full length messenger RNA of the Murine Mammary Tumor Virus (MMTV) harbors an internal ribosome entry site (IRES) within its 5' untranslated region (UTR). Previous studies suggested that the IRES of MMTV requires cellular proteins known as IRES *trans*-acting factors (ITAFs) for its full activity. In this work, we evaluated the potential role as an ITAF for the MMTV-IRES of the polypyrimidine tract binding protein 1 (PTB1), a cellular protein identified as an ITAF for some cellular and viral IRESs. By cotransfecting HEK 293T cells with a plasmid encoding PTB1, along with a bicistronic plasmid encoding a bicistrónic mRNA with the MMTV IRES in the intercistronic region, we established that PTB1 can stimulate the cap-independent translation initiation of MMTV in cells. Using an immunoprecipitation assay we demonstrated that PTB1 protein binds to the MMTV 5'UTR. We conclude that PTB1 protein binds to the MMTV 5'UTR and acts as an ITAF for the MMTV-IRES. Work Supported by FONDECYT 1130270, P09/016-F de la Iniciativa Científica Milenio del Ministerio de Economía, Fomento y Turismo and Beca de Doctorado Nacional from Conicyt.

Protein-DNA recognition: towards a new perspective in the definition, analysis and interpretation of protein-DNA binding specificity

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Protein-DNA binding specificity is currently represented using two-dimensional DNA sequence logos. These logos contain the frequency of occurrence of the four nucleotides at each position of the binding site. Based on this simple representation, which has many drawbacks, particular DNA binding sites are currently described and used to find new motifs that would be recognized by a given protein. It is well known that the use of this methodology leads to many false positives and false negatives when performing complete genome searches.

However, there are some important structural DNA features that are key for protein recognition, which contribution to binding cannot be obtained from this simplified representation. Among these features we find DNA flexibility (key for DNA shape readout) and DNA solvent accessibility through the major and minor grooves (key for DNA base readout). These two features require high order representations at the DNA sequence level to describe accurately DNA molecular properties that are relevant for protein recognition.

In this talk, we will describe our “work in progress” about the development of a new approach based on structural bioinformatics to represent the chemical nature of DNA’s major groove, which would be a key component to understand part of the sequence specificity in DNA recognition through a base readout mechanism by proteins.

As a proof of concept, we will show the preliminary results obtained with four base pair long duplex DNA in the canonical B conformation. These results immediately suggest that the current representation of DNA binding specificity needs urgent modification.

Proteomic characterization of outer membrane vesicles (OMVs) purified from the fish pathogen *Piscirickettsia salmonis*

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Piscirickettsia salmonis is one of the major fish pathogens affecting Chilean aquaculture. This Gram-negative bacterium is highly infectious and is the etiological agent of Piscirickettsiosis. Little is currently known about how the virulence factors expressed by *P. salmonis* are delivered to host cells. However, it is known that several Gram-negative microorganisms constitutively release outer membrane vesicles (OMVs), which have been implicated in the delivery of virulence factors to host cells. In this study, OMVs production by *P. salmonis* was observed during infection in CHSE-214 cells and during normal growth in liquid media. The OMVs were spherical vesicles ranging in size between 25 to 145 nm. A proteomic analysis (LC-MS/MS) for Multidimensional Protein identification technology (MudPit) of OMVs from *P. salmonis* identified 331 different proteins. Thus, several OMVs proteins have been identified to play a key role in *P. salmonis* pathogenesis, as well as some of these proteins form part of the type IV secretion system, flagella and pili structure. Several proteins involved in the process of bacterial conjugation were also identified. These results demonstrated that OMVs are an important contributor to the extracellular proteome and carry highly immunogenic proteins such as flagellins. Therefore, we propose that OMVs purified from *P. salmonis* could be evaluated as a vaccine prototype against Piscirickettsiosis.

FONDAP INCAR 15110027.

Identification of a novel swine H1N2 Influenza A Virus in Chile

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Influenza A Virus (IAV) circulates endemically in swine, human and other species in nature, representing a constant concern to public health and animal production systems worldwide. Although Chile has strong ecological barriers and high stringency regarding livestock across its borders, since 2009 IAV has been consistently detected in pigs. Surveillance of IAVs has therefore been of increasing concern for the swine industry, currently the 6th largest exporter of this meat in the world. We characterized the diversity of swine IAV (SwAIV) and its prevalence in local swine farms. Of 27 farms 21 (78%) were positive at least in one visit. Animals were susceptible to IAV infection at 50-110 days of age. Serological analysis showed 91-59% prevalence to SwIV, with 18-67% positivity to pdmH1N1-2009 and 29-58% to SwH1N2 virus. Genotyping identified the circulation of 4 IAV subtypes in Chile, with at least two strains circulating per production, including: SwH3N2, pdmH1N1-2009 like, SwH1N2, and a reassortant H1N1 containing a human seasonal HA and pdmH1N1-2009 derived NA. We generated and analyzed 69 HA and 50 whole viral genome sequences representative from 2012 to 2014, which revealed multiple human-to-swine pdmH1N1-2009 introductions within the last 4 years. Importantly, we identified novel H1N2 clusters that are unique to Chile and that differ from the H1 clusters seen in North America. This is the first comprehensive study of the diversity and origin of swine IAV in Chile, demonstrating the value of IAV surveillance in South America, a poorly studied region of the world.

The NSs protein of Andes virus is expressed during viral infection and plays roles in eIF2α phosphorylation and stress granules formation.

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Andes virus (ANDV) is a member of the hantavirus genus of the Bunyaviridae family. Andes genome is composed of three negative polarity, single stranded RNA segments denominated according to their sizes as Large (L segment), Medium (M segment) or Small (S segment). These three genomic RNAs (vRNAs) will later be transcribed, and the corresponding messenger RNAs will be translated to give rise to the viral proteins: the RNA dependent RNA polymerase (L protein), the viral glycoproteins (Gn and Gc proteins) and the nucleocapsid protein (N protein) respectively. In 2012, our laboratory identified a new viral protein encoded by the S segment mRNA, the non-structural S segment protein (NSs). The NSs open reading frame (ORF) is located within the N protein ORF, in a +1 position. The NSs initiation codon is recognized by scanning ribosomal 40S subunits recruited in a cap-dependent fashion that have bypassed the upstream N-initiation codon though a leaky scanning mechanism. Expression of the NSs protein was shown in the context of cultured VeroE6 cells infected with Andes virus. In this new study we took advantage of the Syrian hamster infection model, to further validate the expression of the ANDV-NSs protein in the context of a viral infection. This animal model when infected with ANDV develops a disease that resembles the hantavirus cardiopulmonary syndrome, the pathology caused in humans by infection with ANDV. Results show that the NSs protein is expressed in the lungs of infected animals. Next we sought to establish a possible function for the NSs protein. We describe that the expression of the ANDV-NSs protein induces the phosphorylation of the eukaryote translation initiation factor 2α (eIF2α), a key regulator of cellular translation, via the activation of the PKR pathway. We also demonstrate that although this phosphorylation is not enough to inhibit global cellular translation, it is sufficient to induce stress granule assembly. Altogether this study shows that the NSs protein is expressed during a real infection, and that one of its functions is to induce stress granules assembly via phosphorylation of eIF2α.

ORAL SESSIONS 6
PLANT BIOLOGY
Chair: María Josefina Poupin
Co-Chair: María Alejandra Moya-Leon

Biochemical and structural characterization of FcXTH1, a xyloglucan endo-transglycosilase/hydrolase expressed during ripening of *Fragaria chiloensis fruit*

Morales-Quintana, Luis[1]., Mendez-Yañez, Ángela[1]., Beltrán, Dina[1]., Molinett, Sebastian[1]., Campano, Constanza[1]., Herrera, Raúl[1]., Moya-León, Maria Alejandra[1]., [1]Laboratorio de Fisiología Vegetal y Genética Molecular, Instituto de Ciencias Biológicas, Universidad De Talca.

Chilean strawberry (*Fragaria chiloensis*), the maternal progenitor of commercial strawberry (*Fragaria x ananassa*), has emerged as a new berry fruit with excellent organoleptic characteristics. The fast softening of strawberries is a limiting step for their commercialization. Fruit softening has been shown to be related to cell wall disassembling. The cell wall is a dynamic structure that can be modified by different conditions during the cell life cycle. Several enzymes related to this process have been identified in strawberry fruit, and previous studies suggest that XTHs (Xyloglucan endotransglycosidase /hydrolases) might play a key role during ripening of *F. chiloensis* fruit. Two types of XTH activities (transglycosidase XET/ hydrolases XEH) were detected when fruit softening was taking place, coincident with FcXTH1 transcripts increment. The aim of this work was to characterize the FcXTH1 protein. For this, *FcXTH1* was cloned and heterologous expressed in *P. pastoris*. The recombinant purified protein (~36.9 kDa) is active and displayed both XEH and XET activities, with 10-12 times higher XET than XEH activity. The optimal pH was 5.5. Additionally, the stability of the recombinant purified protein at 4 ºC was tested: XET activity is relatively stable at 4 ºC retaining 50% of activity after 5 days, while XEH activity is even more stable, with 78% initial activity after 4 days.To gain insights the mechanism of action of FcXTH1 enzyme at the molecular level, comparative modeling methodology was used to build the structure of FcXTH1. The structure was validated and refined by molecular dynamics simulation. The model obtained display a β-jellyroll–type structure that is typical of GH16 enzyme family that comprises 1 α-helix, 3 3_{10} helices and 15 β-sheets; and a curvature generated by 8 antiparallel β-sheets holds the catalytic D**E**I**D**F**E**FLG motif that is oriented towards the central cavity of the protein. The interaction of a set of putative substrates with the protein was explored using molecular dynamics simulations and molecular docking, finding a better interaction with xyloglucans than cellulose. The data provided allow us to propose that FcXTH1 might be involved in the disassembling of the hemicellulose framework during ripening of *F. chiloensis* fruit.

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The *Daucus carota* phytoene synthase 2 (*PSY2*) promoter: Functional analysis and identification of binding AREB/ABF transcription factors

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Carotenoids are isoprenoid pigments synthesized by all photosynthetic and some non-photosynthetic organisms. In plants, they are synthesized in plastids where they act as accessory pigments in light harvesting and protect cells against photo-oxidation. Carotenoids are precursors of phytohormones such as abscisic acid (ABA), which is involved in dormancy and abiotic stress defense. A key point in carotenoid biosynthesis regulation is the production of phytoene from geranylgeranyl pyrophosphate, reaction catalyzed by phytoene synthase (PSY) and considered the first committed step in the pathway. *Daucus carota*, our model of study, accumulates large amounts of carotenoids, mainly α-and β-carotene, in the modified root only in darkness. In carrot, two paralogs of the PSY enzyme have been described, of which PSY2 is mainly responsible for the biosynthesis of carotenoids in the storage root. Here we show by *in silico* analysisthat the *DcPSY2* promoter, and not that of *DcPSY1*, has three ABA-responsive elements (ABREs), suggesting that this gene may respond to abiotic stress mediated by ABA. Concordantly, only *DcPSY2* expression is induced by ABA in carrot seedlings. The functional characterization of the *DcPSY2* promoter in transgenic *Nicotiana tabacum* indicates that the complete *DcPSY2* promoter fused to *GFP*, but not promoters with fewer ABRE elements, activate the transcription of the reporter gene in response to ABA. Furthermore, after *in silico* selection of three carrot ABA responsive transcription factors (AREB/ABF), we determined through a yeast one-hybrid assay that they bind to the *DcPSY2* promoter and activate the transcription of reporter genes.

Transcriptional control of pollen-specific genes in *Arabidopsis thaliana* by GT-4 and STEP1.

Parra, S[1]., León, Gabriel[2]., [1]Centro de Biotecnología vegetal, Ciencias Biológicas, Universidad Andrés Bello. [2]Sexual Plant Reproduction, Ciencias Biológicas, Universidad Andrés Bello. (Sponsored by Founded By Fondecyt 1120766 UNAB DI-845-15/I)

To achieve a successful fertilization, pollen grains must germinate and elongate a pollen tube to carry the sperm cells to the embryo sac, a process that requires the coordinated integration of several pathways. In our laboratory we have characterized 4 RECEPTOR LIKE CYTOPLASMIC KINASES (RLCKs), which contribute to several aspects of pollen and pollen tube function. In addition, we have identified two putative regulatory proteins: GT-4 – a potential transcriptional activator– and STEP1, a ribonucleoprotein possibly involved in the transcriptional repression of RLCKs. GT-4 has been reported as a transcriptional activator and analysis of our laboratory suggest that participates as an enhancer of the expression of a subset of specific pollen genes. On the other hand, analysis of pollen grains from *step1* mutants exhibit a great change in the rheological properties of the pollen tube, defects in pectin deposition and abnormal branching growth of the pollen tubes compared to wild type plants. Strikingly, *in vitro* pollen germination of *step1* mutants revealed the presence of fragments of pollen tubes released during the elongation process, a phenotype that suggest severe anomalies in the pollen tube structure and signaling processes associated ti pollen tube growth. Our analyses show that lack of expression of the ribonucleoprotein STEP1 mainly affects some pectin methylesterases genes (PMEs) which are crucial in the process of cell wall deposition at the tip of the pollen tube, affecting the ability of these pollen grains to carry their sperm cells and achieve the fertilization process.

Iron localization in Arabidopsis seeds: Connecting nutrition and provasculature patterning

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The mechanisms of iron uptake in plant roots are now well described. However, those governing the distribution to organs, cells and organelles targets are still very poorly understood. The development of methods that allow the visualization of iron pools in the plants has been key in the significant progress observed in this field regarding the characterization of new genes involved in iron distribution in plant tissues. Recently, a histochemical iron staining method (Perls/DAB) has been developed for plant tissues. Thanks to this approach, it has been possible to identify new pools of iron in plants. For instance, we described that iron accumulates in the vacuoles of endodermis during seed maturation. Considering that iron localizes in the endodermis, which surround the provasculature, we hypothesize that changes in the complexity of vein pattern in embryo may affect total iron content in Arabidopsis seeds. Modifying iron content of seed for human consumption (biofortification) is a possible alternative to combat iron deficiency, which is a serious public health problem and a major concern for the World Health Organization (WHO). Here we will describe where iron accumulates in plants and how changes in the cotyledon vein network of embryos may affect total iron content in Arabidopsis seeds.

Flavonols and Auxin: A gravitropic relationship

Ramos, Patricio[1]., Guajardo , Joselin[1]., Moya-León, María[1]., Herrera, Raúl[1].,[1]Laboratorio de Fisiología Vegetal y Genética Molecular, Instituto de Ciencias Biológicas, Universidad De Talca. (Sponsored by This Work Was Supported By Fondecyt 11121170, 1120635 And Anillo ACT-1110)

Stem reorientation in response to inclination is a widely studied phenomenon, but in trees the molecular mechanism is still unknown. The most accepted theory that explains this differential response is auxin redistribution in stem tissues. 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 inclination through transcriptional, chemical and microscopic approaches. 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 an induction in response to stem inclination; higher expression levels were recorded at the basal zone and in the upper half of the stem. Concomitant with these expression profiles, 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. According with the expression pattern of an auxin-gene marker, its distribution was evidenced in the lower half of inclined stem by an immuno-specific assay. Results suggest a concerted activation of genes and metabolite accumulation in the upper side of inclined stem that generate a misbalance in local auxin distribution. This molecular mechanism could be triggering stem reorientation with a subsequent abnormal wood formation in the lower side of inclination-stressed plants.

NEW MEMBERS SESSION
Chair: Sergio Lavandero
Co-Chair: Alfredo Criollo

Generating a Non-Toxic Synthetic Prion that can Delay or Prevent Prion Disease In Vivo

Diaz-Espinoza, Rodrigo [1]., Morales, Rodrigo[1]., Concha-Marambio, Luis[1]., Moreno-Gonzalez, Ines[1]., Moda, Fabio[1]., Soto, Claudio[1]., [1]Neurology, Medicine, University of Texas .

Transmissible Spongiform Encephalopathies (TSEs) are fatal neurological disorders caused by proteinaceous infectious agents termed prions. These particles are composed of an aberrantly folded protein (PrPSc) that self-propagates in the brain of infected individuals by converting the normally folded prion protein (PrPC) into PrPSc. This infection process is typically associated to extensive brain degeneration and motor impairment. The unconventional nature of prions has occluded the development of efficient therapeutics against TSEs. Here we report that a one- time prophylactic inoculation of prion-infected animals with a non-toxic misfolded recombinant PrP can delay the onset of the disease and in some cases completely prevent the development of clinical symptoms. Furthermore, this treatment caused significant changes in the pattern of PrPSc deposition in the brains of animals that still succumbed to the disease. These results provide new insights for a mechanistic understanding of the relationship between prion toxicity and infectivity in TSEs that can lead to the development of a radically novel antiprion therapeutic approach.

Hypoxia-inducible factor-1, Epithelial-to-Mesenchymal-Transition and Chemotaxis.

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Hypoxia-inducible factor-1 (HIF-1) is essential in response to hypoxia. *In vitro* assays have show that HIF-1 directly regulates key regulators in cancer cell progression and migration. HIF-1 is also expressed during normal embryonic development. We decided to test whether Hif-1 is required during this process, focused on the development of the neural crest, a highly migratory embryonic cell population. Inhibition of Hif-1α by antisense morpholinos in *Xenopus* or zebrafish embryos led to complete inhibition of neural crest cells migration. Our data show that Hif-1α controls the expression of *Twist* and *Snail*, which in turn represses E-cadherin during epithelial-to-mesenchymal transition (EMT) of neural crest cells. Thus, Hif-1α allows cells to initiate migration by promoting the release of cell-cell adhesions. Additionally, Hif-1α controls chemotaxis towards the chemokine SDF-1 by regulating expression of its receptor Cxcr4. Our results point to Hif-1α as a novel and key regulator that integrates EMT and chemotaxis during migration of neural crest cells.

TDP-43 loss of cellular function through aggregation requires additional structural determinants beyond its C-terminal Q/N prion-like domain.

Budini, M[1,2]., Valentina, Romano[2]., Quadri, Zain[2]., Buratti, Emanuele[2]., Baralle, Francisco[2]., [1]Facultad de Odontología Universidad De Chile. [2]Molecular Pathology Lab International Centre For Genetic Engineering and Biotechnology.

Introduction: TDP-43 aggregates are the neurohistological landmark of diseases like Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD). Their role in the pathogenesis of these conditions is not yet clear mainly due to the lack of proper models of aggregation that may allow the study of the mechanism of formation, their interactions with other cellular components, and their effect on the cell metabolism. **Methodology**: we have used tandem repeats of the prion like Q/N-rich region of TDP-43 fused to full length TDP-43 protein to trigger aggregate formation in neuronal and non-neuronal stably transfected cell lines. **Results:** these aggregates are able to sequester endogenous TDP-43 depleting, in some cases, the TDP-43 nuclear levels, and inducing loss of TDP-43 function at evaluated by the pre-mRNA splicing of the gene target POLDIP3. No apparent direct cellular toxicity of the aggregates seems to be present beyond the lack of functional TDP-43. Additionally we identified the N terminus sequence of TDP-43 as the domain that enhances its interaction with the aggregates and its insolubilization. **Conclusions:** to our knowledge, this is the only system that achieves full functional TDP-43 depletion with similar effects to RNAi depletion. As a result, this model will prove to be useful to investigate the loss-of-function effects mediated by TDP-43 aggregation within cells without affecting the expression of the endogenous gene. These data show for the first time that cellular TDP-43 aggregation can lead to total loss of function and to defective splicing of endogenous genes targets for TDP-43.

Influence of the infectious pancreatic necrosis disease virus in the synthesis of cellular proteins.

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Infectious pancreatic necrosis virus (IPNV) is a pathogen that affects salmonids causing high economic losses in Chile and worldwide. This unenveloped virus possesses two segments of non-capped nor-polyadenylated double strand RNA as genome. Noteworthy, each 5'extreme of both RNA segments are covalently bonded to the viral protein VPg, which acts as a primer in the RNA synthesis. When Vpg is not bonded to the RNA, is called VP1, and it is the RNA-polymerase RNA-dependent that synthetizes the new RNA. The replicative cycle of this virus is not well known. It is particularly interesting to evaluate how its mRNA, which contains a viral protein bonded to its 5' extreme instead of Cap, is translated. In this work, we studied the translation of viral mRNAs, and the effect of viral proteins in cellular protein expression, showing that IPNV exerts a marked influence on Cap-dependent translation, allowing the synthesis of their own proteins.

POSTERS SESSION

Posters Wednesday, September 23

1) RNA seq as a tool to study the effect of handling stress in the expression of genes associated with the immune response of red cusk-eel (*Genypterus chilensis*)

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Introduction: The red cusk-eel is an endemic species, which has been proposed to diversify the national aquaculture. In intensive fish farming have been described numerous factors that can affect the homeostasis of fish species, one of these is the handling stress.. In this work we studied the effect of handling stress on the immune response of *G. chilensis* by identifying differentially expressed genes that are related to the innate or adaptive immunity of this specimen.

Material and Methods: Total RNA was extracted from head kidney of juvenile red cusk-eel under control and stressed conditions. RNA samples were sequenced using Miseq (illumina) technology in the Centro de Biotecnologia Vegetal (UNAB). Subsequently, through the CLC genomic workbench software (version 7.03) the reads obtained were processed and *de novo* assembly and later differential expression analysis was performed.

Results: We obtained a transcriptome of 85.081 contigs with an average size of 840 bp with a coverage of 101 X. Moreover, we identified the presence of 1056 contigs are differentially expressed. By analyzing the stress condition with respect to the control condition it was observed that 543 contigs are over-expressed, while 513 are suppressed., Further preliminarily, we found the presence of genes immune relevance as *il-1ϐ, tnf-α, cxc8, il-10, trl-1, tlr-2, tlr-5, c3, c5, casp-1, casp-3a, mhc-1, mhc -2*, among others.

Discussion: Our results will provide a rich source of information for future studies, possibly aimed at improving the productivity of intensive red cusk-eel farming.

Founded by CONICYT/FONDAP/15110027 and FONDECYT/1130545

2) Dissecting the functional roles of the conserved NXXE and HXE motifs in the ADP-dependent glucokinase from *Thermococcus litoralis*

Abarca, M. J[1]., Ramírez-Sarmiento, César [1]., Rivas-Pardo, Jaime[1]., Guixé, Victoria[1]., [1]Departamento de Biología, Ciencias, Universidad De Chile. (Sponsored by FUNDING: FONDECYT 1150460.)

The archaeal glucokinase from *Thermococcus litoralis* (*Tl*GK) catalyzes the ADP-dependent phosphorylation of glucose to glucose-6-phosphate, in contrast to its ATP-dependent counterparts. Its catalytic mechanism relays on highly conserved motifs: the GXGD motif, involved in the activation of the phosphoryl acceptor substrate; and the NXXE and HXE motifs, related to binding of two divalent cations. One cation is present in the true catalytic substrate, MgADP, while the second is inhibitory. We assessed the role of residues E279 and E308, belonging to the HXE and NXXE motifs, on the kinetic parameters of *Tl*GK. In comparison to the wild-type enzyme, the E308Q mutant showed 100-fold increase on Km for MgADP and activation by free Mg^{+2}. Surprisingly, the E279L and E279Q mutants were still inhibited by free Mg^{+2} but showed 30 and 400-fold increase on Km for glucose, respectively. Considering this effect and the sequentially ordered kinetic mechanism proposed for *Tl*GK where MgADP binds first, we decided to evaluate whether the HXE mutants affected the binding of glucose onto the MgADP-*Tl*GK complex, as ascertained by measuring the increase in intrinsic fluorescence of *Tl*GK in the presence of the MgADP analog, AMP-AlF_3. Our results showed that wild-type *Tl*GK and E308Q bind glucose with similar affinities, having dissociation constants (Kd) of ~127 µM. In contrast, the HXE mutants exhibited a 87-fold increase in Kd. The excellent agreement between the estimated Kd and Km values for glucose suggest that the HXE motif is fundamental for binding and stabilization of glucose during the formation of the ternary complex.

3) Pipeline to identify the toxic factor(s) released by diverse ALS-astrocytes that induce pathogenesis and death of motoneurons.

Abarzúa, Sebastián[1]., Rojas, Fabiola[1]., Cortes, Nicole[1]., Martínez, Pablo[1]., Aguilar, Rodrigo[1]., Almeida, Sandra[2]., Kähne, Thilo[3]., Wyneken, Ursula[4]., Varela-Nallar, Lorena[1]., Gao, Fen-Biao[5]., Montecino, Martín[1]., Van Zundert, Brigitte[1]., [1]Center for Biomedical Research, Biological Sciences & Medicine, Universidad Andrés Bello. [2]The J. David Gladstone Institutes, Buck Institute for Research on Aging University of Massachusetts Medical School. [3]Institut für Experimentelle Innere Medizin Otto-von-Guericke-Universität Magdeburg. [4]Laboratorio de Neurociencias Universidad De Los Andes.[5]Department of Neurology University of Massachusetts Medical School.

Introduction: Amyotrophic lateral sclerosis (ALS) is a devastating paralytic disorder in which pathogenesis initiated during early development leads to degeneration of motoneurons in adulthood. Published studies by us and others have shown that non-cell autonomous processes are involved as astrocyte conditioned media (ACM) generated by astrocytes derived from mice expressing mutant human superoxide dismutase 1 (hSOD1^{G93A}) or transactive response DNA binding protein 43 (hTDP43^{A315T}) release of a neurotoxic factor(s) and cause pathogenesis and death of wild-type motoneurons. Neither the toxic factor(s) nor its primary target on motoneurons has been identified. Here we set-up a platform to identify in an unbiased manner the neurotoxic factor secreted by diverse ALS astrocyte models. **Methods:** Mouse-primary-astrocytes wereprepared from P1-2 control and transgenic mice that carry human SOD1^{WT}, SOD1^{G93A} or TDP43^{A315T}. Human-induced-astrocytes were differentiated from human induced pluripotent stem cells (hIPSCs) generated from adult control subjects or ALS patients (TDP43^{A90V}). To analyze toxicity mediated by ALS-astrocytes, wild-type primary rat ventral spinal cord cultures (VSCCs) were incubated with mouse-primary-ACM or co-cultured with human-induced-astrocytes; ROS production, c-Abl phosphorylation and motoneuron survival were tested. For quantitative mass spectrometry-based proteomics, mouse-primary-ACMs (SOD1^{WT} and SOD1^{G93A}) were analyzed by MALDI target plate and mass spectra analyzed by the Mascot search engine. For the identified candidate protein/peptides, lentivirus-mediated RNAi was employed to knock-down expression of their coding genes in mouse-primary-astrocytes and ACM-hSOD1^{G93A} was tested for toxicity. **Results:** Application of ACM-SOD1^{G93A} and ACM-TDP43^{A315T} to VSCCs led to strong ROS production and c-Abl phosphorylation, as well as to a robust reduction in motoneuron survival. Similarly motoneuron toxicity was obtained with TDP43^{A90V} human-induced-astrocytes. Mass spectrometry analysis of mouse-primary-ACMs detected various interesting proteins/peptides, and importantly, knocking-down the expression of two candidates in SOD1^{G93A}-containing astrocytes produced ACM that was significant less toxic to motoneurons. **Discussion:** Additionalmass spectrometry is ongoing with new ACM samples to identify additional secreted factors. Our established experimental set-up will allow to validate candidate factors in both mouse and human ALS models.

4) Analysis of confinement-stress transcriptomic response on the skeletal muscle of the fine flounder *(Paralichthys adspersus)*

Aedo, J E[1]., Mendez, K[2]., Vizoso, P[3]., Bastías, M[3]., Meneses, C[3]., Zuloaga, R[2]., Valenzuela, C[2]., Valdés, J A[1]., Molina, A[2]., [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]Centro de Biotecnología Vegetal, Facultad de Ciencias Biológicas , Universidad Andrés Bello.
(Sponsored by Funded By CONICYT/FONDAP/15110027 And FONDECYT /1130545)

Introduction: *Paralichthys adspersus* has been considered one of the endemic species of greatest farming potential in Chile. Recently, we generated a reference transcriptome of *P.adspersus* with a significantly amount ofannotated genes. Using that information as a reference, in this work we evaluated the effects of confinement stress on the gene expression of *P. adspersus* skeletal muscle. **Material and Methods:** RNA from skeletal muscle of confinement-stressed and unstressed *P.adspersus* were collected and sequenced by illumina technology. Raw data obtained were filtered and high-quality reads were aligned onto *P.adspersus* reference transcriptome using bowtie 2.0 software. Finally we detected transcript with differential expression using edgeR program. **Results:** Sequencing generated 83,584,178 raw reads with an average length of 185bp. A total of 78,505,811 high-quality reads were aligned onto reference transcriptome available of *P.adspersus.* We identified 317 transcripts with differential expression during confinement stress in the skeletal muscle of *P.adspersus* (Fold of change > 2, FDR pvalue <0.001). Among these transcripts we found important atrogenes, structural proteins, ion channels, metabolic enzymes and transcription factors. **Discussion:** Our results indicate that confinement stress modulates the expression of genes associate to skeletal muscle growth in *P. adspersus*. This study allows us to identify new expressed genes associated to confinement-stress stimulus on the skeletal muscle of this promissory farming fish.

5) Reassessing cAMP-dependent Protein Kinase A and Phosphodiesterase inhibitors role in the *Neurospora crassa* circadian clock regulation

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Circadian rhythms are widely conserved in all kind of organisms, allowing them to anticipate environmental changes. *Neurospora crassa* has been paramount in the molecular characterization of these rhythms, and of the central oscillator underlying eukaryotic systems. One of the proposed regulatory mechanisms for the *N. crassa* circadian clock is related to the amply studied cAMP intracellular signaling pathway; previous publications describe mutant organisms for PKA -central element in this pathway- as lacking circadian rhythms according to phenotypical assays, suggesting this protein as fundamental for the normal functioning of the circadian oscillator. Since PKA is one of the central elements in growth and regulation of development in Neurospora, main aspects evaluated in circadian phenotypical assays, we re-evaluated how cAMP signaling pathway participates in the circadian clock using techniques that allow direct measurement of the central oscillator. Using bioluminescent reporters under the control of different clock *cis* elements, we analyzed mutant strains for each element of the aforementioned pathway, observing circadian rhythms similar to those of the wild type strain in each case. With the same techniques we evaluated the effect of three phosphodiesterase inhibitor drugs; caffeine, theophylline and aminophylline. For these three drugs period lengthening of the *Neurospora* circadian rhythms has been described and was observed, even when analyzed in all cAMP signaling pathway mutants, through luciferase reporters. This suggests that the effect of these drugs is due to a different mechanism of action that the one previously hypothesized. MNNFISB NC120043, FONDECYT 1131030.

6) The Sall2 transcription factor participates in the oxidative stress response.

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Cells can respond to stress in various ways, they can adapt and survive or initiate cell death mechanisms to eliminate damaged cells. In this context, particular proteins lead the cell response to specific stresses, examples are the heat shock proteins (HSPs), or transcription factors like p53, among others. Recently, we characterized the Sall2 transcription factor as a stress-response molecule against genotoxic stress. We demonstrated that Sall2 plays a key role in promoting the expression of proapototic proteins Bax and Noxa, which are necessary for the cell death response during DNA damage. On the other hand, unpublished data from our laboratory show that Sall2-deficient mouse embryo fibroblasts ($Sall2^{-/-}$ MEF) have increased cell death under metabolic stress. Several insults, including metabolic stress, converge in the generation of reactive oxygen species (ROS) and, consequently, oxidative stress. Thus, we are now investigating whether Sall2 participates in the cellular response to ROS. By Western blot and qPCR analysis, we found that Sall2 levels increase after exposure to hydrogen peroxide. In addition, we identified putative Sall2 binding sites in NADPH oxidase 1 activator (NOXA1), a protein required for superoxide generation. Promoter- reporter assays indicate that Sall2 regulates the NOXA1 promoter. Altogether, our results suggest that Sall2 participates in the oxidative stress response induced by hydrogen peroxide by regulating the expression of NOXA1. We are further investigating the role of Sall2 in NOXA 1 expression and in the cellular response to oxidative stress.
FONDECYT 1151031

7) L-valine production: From a scientific discovery in Antarctica to a Biotech Start-UP company.

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Amino acids are industrially relevant due to their use on feed, food, poultry and Pharma. Among the essential amino acids, L-valine has gained attention because is the fifth limiting amino acid on animal nutrition and during 2014 the L-valine global market was USD 2 billions.

Almost all L-valine production is carried out by fermentation using engineered strains of Corynebacterium glutamicum. However, this microorganism is not capable of producing L-valine under oxygen deprivation because of a shift of the central metabolism towards lactate. Moreover, C. glutamicum induction of secretion requires high concentrations of Tween 40 or 60, generating loses of up to USD 5 millions per year.

Aminotec has designed a new method for fast screening of amino acid producing bacteria, allowing us to identify PCN1, a Gram negative Antarctic isolate that secretes 4g/L of L-valine on M9 media amended with 3% glucose and no addition of Tween 40 or 60. Furthermore, this microorganism is able to produce L-valine under oxygen deprivation.

The genome sequence of PCN1 shows that this microorganism is an Enterobacteriaceae, containing several genomic copies of the AHAS enzyme that catalyzes the first step on the conversion of pyruvate to L-valine. Based on genomic and metabolic characteristics of PCN1, we are now generating mutant strains to improve amino acid production. Aminotec's aim is to reduce the operational costs of L-valine production by 40%.

Supported by: Grant UNAB DI-476-14/R to DEA, CORFO 15SUP-39021 Aminotec.

8) Expression of Herpud isoforms during osteoblast and osteoclast differentiation.

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Introduction: Bone is a dynamic tissue whose integrity is the result of balance among synthesis by osteoblasts and resorption by osteoclasts. The differentiation of osteoblasts and osteoclasts are regulated by several mechanisms involved in protein control quality. Osteoblastogenesis requires the activation of proteins involved in endoplasmic reticulum (ER) stress response. Whereas, resorptive phenotype of osteoclast requires activation of autophagy. Herpud1/2 are ER-membrane integral proteins. Herpud1 is up regulated in response to ER-stress, plays a role in ERAD and autophagy. Whereas the function of Herpud2 is unknown.

The aim of our work was determinate the levels of Herpud1/2 during the osteoblast and osteoclast differentiation.

Methodology: Pre-osteoblast MC3T3 and pre-osteoclast RAW264.7 cell lines was treated with different ER-stress and autophagy inducers and differentiated for 3, 7 or 14 days. The differentiation was evaluated through increase in calcium deposits, ALP or TRAP activity and expression of differentiation markers. The levels of Herpud1/2 were determinated through western-blot.

Results: Our results shown, an increase in Herpud1 in response to ER-stressors and a reduction of Herpud2 in response to autophagy inducers in MC3T3. In initial stages of differentiation (12, 24 h) both proteins are increased in both cellular lineages, but during osteoblastogenesis was observed an increase in Herpud1 levels, in contrast with osteoclastogenesis, where was observed a reduction of this protein. **Conclusions:** This results lets us speculate that Herpud1 has a differential role in osteoblasto- and osteoclastogenesis, and could be a candidate to regulate the bone turnover.

9) The C-terminal tail of yeast Hmo1 is essential for its interaction with ATP-dependent chromatin remodeling complexes

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Chromatin structure is an essential component in the regulation of all processes that require DNA-protein interactions, such as transcription. ATP-dependent chromatin remodeling complexes are key factors in chromatin remodeling. SWI/SNF and ISWI are complexes belonging to the family of remodeling enzymes. Certain High Mobility Group (HMG) proteins are able to stimulate ATP-dependent chromatin remodeling activity. We have previously shown that the yeast Nhp6A, Nhp6B and Hmo1 proteins (HMGB family members) stimulate the sliding activity of ySWI/SNF and that only Hmo1 stimulates other biochemical outcomes of ySWI/SNF activity. Additionally, the sliding activity of ISW1a is only stimulated by Hmo1. These differential stimulatory effects of Hmo1 appeared to be dependent on the presence of its C-terminal tail, which contains a stretch of acidic and basic residues. Considering these results, we wanted to determine whether Hmo1 physically interacts with SWI/SNF and ISW1a complexes, and whether the presence of the C-terminal tail is important for these interactions. To do this, we performed GST pull-down assays to analyze the interaction between Hmo1 with the SWI/SNF and ISW1a complexes. Interaction of Hmo1ΔCt (Hmo1 lacking its C-terminal tail), Nhp6A and Nhp6B with these complexes will also be tested. Additionally, we analyzed whether Hmo1 stimulates the recruitment of SWI/SNF by the chimeric transcription factor Gal4-VP16 to a mononucleosome, by performing EMSA assays. Our results show that Hmo1 physically interact with both SWI/SNF and ISW1a complexes and is also able to enhance the recruitment of SWI/SNF by Gal4-VP16 to a mononucleosome probe.

10) CyDiv, a protein involved in divisome assembly in *Anabaena* sp. PCC7120.

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Bacterial cell division is a fundamental and complex process being that requires coordinated mechanisms to elongation and septal formation in the middle of the cell. The most of the research in cyanobacterial division have been focused on identifying proteins that interact specifically with the FtsZ protein, but this also revealed that machinery of division presents differences in composition and regulation compared to the division models of gram-negtive and gram-positive. Previous studies carried out in our laboratory, it was determined a gene common to all filamentous cyanobacteria (*cyDiv*) which encodes a protein with a transmembrane and coiled-coil domains. The mutant phenotype of this presents effect in cell division. To determine how the protein CyDiv contributes to divisome ensemble in filamentous cyanobacteria Anabaena sp. PCC7120, we analyzed phenotypic variations under different transcription levels controlled by copper-inducible promoter and determine the localization protein by immunofluorescence. Furthermore, was evaluated association of CyDiv with other protein involved in cell division by BACTH system. We examined cellular localization and protein interaction suggest that CyDiv is essential for cell division in Cyanobacteria and perform its role through interaction with elements of divisome. Alteration of *cyDiv* transcription in *Anabaena* sp. PCC7120 led to effects in the Z-ring positioning and cell wall damage triggering cell lysis. We show that this protein localized depending of time of division from pole to cell centre and determine that this protein has similar topology of the protein from *E. coli* FtsB.

11) Genome-wide transcription induced by Wnt/β-catenin signaling in neuronal cells as a model for prevalent neurological disorders.

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Introduction: We proposed earlier that Wnt/β-catenin signaling is a functional, temporal and positional candidate to understand complex prevalent neurological disorders, including autism and Alzheimer's disease. Here we begin to dissect on a genome-wide basis the genetic program controlled via β-catenin and LEF1/TCF transcription factors in hippocampal cells, which are affected by the onset or progression of these disorders. **Material and Methods:** Total RNA was extracted from HT22 mouse hippocampal cells incubated with or without purified Wnt3a protein (200 ng/mL; 4 h). RNA (RIN value > 9) was reverse-transcribed into cDNA and processed using the Illumina TruSeq Stranded mRNA kit to generate 250 pb size libraries. Libraries were sequenced using the Illumina MySeq (depth > 12 million pair-end reads). Raw reads were aligned to Mouse genome (mm10) usingBioconductor. Candidate genes were statistically analyzed by DESeq and subjected to pathway analysis (Panther v10.0). **Results:** From a total of 8,718 confident calls (after QC and variance normalization), there were 95 and 84 up- or down-regulated genes, respectively (> 2 fold-change) after Wnt3a-treatment. Ontological analyses revealed that Wnt (49/521; components/subfamilies, respectively), interleukin (36/122) and glutamate signaling (metabotropic and ionotropic; 41/177) pathways were over-represented categories in upregulated genes. Similar analyses in downregulated genes showed that Alzheimer's disease (31/91), Toll receptor (46/84) and inflammatory signaling (58/366) were detected. Candidate genes are currently being validated through qPCR. **Conclusions:** Our results indicate that Wnt3a is able to modulate a rapid global transcriptional program involved in neuronal synaptic function and metabolism. **Funding**: FONDECYT 3130509 and 1140353, FONDAP 1509000.

12) A-to-I ADAR1 transcriptome editing in breast cancer disease.

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The double stranded RNA-specific adenosine deaminase (ADAR1) catalyzes the deamination of adenosine in pre-mRNAs, non-coding RNAs and matures mRNAs resulting in important stability, function and expression changes of its targets. Editing unbalance has been demonstrated in several diseases, including cancer, where increased editing levels are strongly correlated with the progression of the disease. However, the expression and activity of ADAR1 in breast cancer is not well characterized. Gene expression data from The Cancer Genome Atlas (TCGA) cohort, which includes 1214 breast cancer samples, was analyzed and a novel strategy to call single nucleotide changes in the RNA, in order to evaluate the editing changes present in these patients, was implemented. In addition, the expression levels of ADAR1 and the editing levels of ADAR1 targets were measure using RT-qPCR and RESSqPCR in three breast cancer cell lines (MCF10A, MCF7 and ZR-751). The analysis of expression data from TCGA showed a significant overexpression of ADAR1 in tumors compared to normal samples. These changes also correlated with the editing and expression levels of ADAR1 in the breast cancer cell lines analyzed. In addition, the bioinformatics analysis revealed a significant enrichment of A>G transition in the tumor compared to normal samples, showing significant editing changes in mRNAs associated to the DNA replication and cell cycle process. Impending work is needed to elucidate the function of the editing changes mediated by ADAR1 and their relation with the breast cancer progression.

13) Identification and characterization of virulence effectors Dot/Icm-related in the fish bacterial pathogen Piscirickettsia salmonis

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Piscirickettsia salmonis is a gram-negative bacterium, etiological agent of Piscirickettsiosis, an infectious disease that affects the global salmon farming since the late 80's. *P. salmonis* is facultative intracellular organism, not mobile and is able to infect and survive in fish macrophages. Recent reports confirm that this agent have a Dot/Icm Secretion System, homologue to the described for *Legionella pneumophila* and *Coxiella burnetii*.

This work focused in make an *in silico* and molecular characterization of putative virulent effectors (Dot/Icm substrates) in *P. salmonis*, using as reference those described for *L. pneumophila* and *C. burnetii*. To do so, 294 reference Dot/Icm substrates of *L. pneumophila* and *C. burnetii* were screened in the genome of *P. salmonis* LF-89, using a local BLASTP of the RAST server in order to find homologies. Was possible identify 5 proteins of *P. salmonis* with homologies to the reference Dot/Icm substrates, which contains eukaryotic conserved domains (Serine-threonine kinase C, SEL-1, U-Box and ankyrins repeats), a typical feature of Dot/Icm-related virulence effectors. Additionally, the 5 *P. salmonis* proteins contain the classic Dot/Icm secretion signal in the C-terminal (E-Block, region with negative residues, hydrophobic or proline residue at position analyzed -3 or -4).

Finally, the nucleotide sequence of the five Dot/Icm substrates was cloned into pYES3-CT vector and the proteins were expressed in *Saccharomyces cerevisiae*, in order to evaluate their over eukaryotic cells.

14) STUDY OF TACROLIMUS ON CHEMOSENSITIZATION OF THE GLIOBLASTOMA STEM LIKE CELLS

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Introduction. The glioblastoma stem like cells (GSCs) have been associated with the phenotype of multiple drug resistance (MDR). This cell population exhibits a high expression of MDR transporters, where MRP1 facilitate the removal of drugs. We have detected that the immunosuppressant tacrolimus (FK506) can inhibit the expression and activity of MRP1 in bulks cells, but we do not know what happens with the GSCs. Our objective was to evaluate the chemosensitizer effect of FK506 on GSCs. **Methods.** To evaluate the effect of FK506 on the expression and activity of MRP1 in GSCs, qPCR western blot and CFDA retention assay were conducted. Moreover, cell viability of GSCs incubated with FK506 alone or in combination with vincristine was measured by MTT assay. *In vivo* experiments were performed by inoculating Sprague-Dawley rats subcutaneously with GSCs to generate a tumor. Tumor growth was monitored every 3 days for 10 days and then treated with PBS, FK506 (0,32mg/Kg/day), vincristine (0,1mg/Kg/day) or FK506 together vincristine. **Results.** The gene and protein expression of MRP1 did not change by FK506, however, the MRP1 transporter activity decreased up to 24± 0.06fold, which could indicatea potential benefitin usingthis drug as a tumor chemosensitizer. Furthermore,FK506+vincristine treatment decreases both cell viability in vitro and the tumor growth*invivo* (about 34%). **Conclusion.** Since FK506 inhibits the activity of MRP1 *in vitro* and the tumor growth in vivo, it could be used as a chemosensitizer for the chemotherapeutic treatment of glioblastoma.

15) HMGA1a and HMGA1b proteins enhance the remodeling activity of the ySWI/SNF complex

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The remodeling of chromatin is involved in several DNA-related processes, including transcriptional regulation. SWI/SNF is a complex with ATP-dependent chromatin remodeling activity. This complex can be targeted to specific gene regulatory regions by transcription factors. Its activity can be stimulated by elements such as histone acetylation and some HMG proteins, which are divided in three families: HMGA, HMGB and HMGN. All these proteins have DNA binding activity, but only HMGA display a sequence-specific binding activity. To date, stimulation of SWI/SNF activity has only been demonstrated for HMGB proteins. Considering the sequence-specific DNA binding activity of HMGA and that several gene promoters contain binding sequences for this protein, we wanted to analyze whether this HMG protein can also stimulate SWI/SNF activity and its recruitment by transcription factors. To carry out these analyses we performed remodeling and binding assays using mononucleosomes reconstituted *in vitro*, which contain an HMGA binding site in an extranucleosomal DNA region (linker DNA). These probes also contain a Gal4 binding site adjacent to the HMGA cognate site. The assays were visualized by electrophoresis in non-denaturant gels. First, we analyzed HMGA1a and HMGA1b binding activity to mononucleosome probes containing different HMGA binding sequences, selecting the probes giving the strongest binding. Our remodeling assays showed that HMGA1a and HMGA1b stimulate SWI/SNF sliding activity. Stimulation of SWI/SNF binding to the nucleosome and recruitment of this complex by the chimeric factor Gal4-VP16 will be also analyzed. Our results uncover new mechanisms related to the participation of HMGA proteins in transcriptional regulation.

16) Filovirus-derived endogenous viral elements in the genome of a South American rodent

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Viral infection of gametes or early embryos can lead to viral genes or genomes becoming integrated into nuclear DNA and inherited as host alleles. These insertions are referred to as *endogenous viral elements* (EVEs). In animals most EVEs are derived from retrovirus, due to its replication strategies that include integration into the host chromosomes. EVEs derived from viruses that use other replication strategies are less common, but EVEs derived from parvo-, bunya-, filo-, rhabdo-, orthomxyo-, flavi- and reoviruses have been described. Filoviruses are non-segmented (-) strand RNA viruses that infect mammals, among the members of this family are the genus *Ebolavirus, Margburgvirus* and therecently added *Cuevavirus*. EVEs derived from Filoviruses have been found in the genomes of several African, Asian and Australian animals. Using *in silico* strategies we identified an EVE derived from filovirus in the genome of the long-tailed chinchilla (*Chinchilla lanigera*) a rodent native to South America. This finding was confirmed by PCR and sequencing of chinchilla DNA. This is the first description of an EVE derived from filovirus in an indigenous South American animal. These findings demonstrate that filoviruses have circulated among South American mammals in the past,. Sequence-based surveillance can help determine whether exogenous filoviruses continue to circulate in South American mammals, and whether there is a risk of zoonotic transfer from potential vector species in this region, such as vampire bats (*Desmodontinae*).

17) Improving the catalytic efficiency of H. rufescens's β-glucuronidase towards codeine-6-glucuronide.

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The enzymatic hydrolysis of drugs conjugated with glucuronic acid is the first step in drug analysis from biological samples. Yet, the efficiency of these enzymes is low for several drug conjugates, among them codeine-6-glucuronide. Thus, toxicology labs across the world are hoping for more efficient β-glucuronidases. Recently, the Chilean company Kura Biotec started commercializing *H. rufescens'* β-glucuronidase, which more efficiently hydrolyzes codeine-6-glucuronide. Given that this is a naturally occurring enzyme, however, Kura cannot protect it under industrial property, and thus, here we designed rational mutants of this β-glucuronidase that increase its catalytic efficiency towards codeine-6-glucuronide and are thus patentable subject matter.

First, starting from a nucleotide sequence for *H. rufescens'* β-glucuronidase we assembled from public RNA sequence data, we built a homology model of the enzyme based on the human homologue whose structure is known. Special care was taken to use the most catalytically relevant protein chain deposited in the PDB as template. Then, using protein-ligand docking we rationalized the higher affinity of this enzyme towards codeine-6-glucuronide. Using an in house automated pipeline we then performed *in silico* saturation mutagenesis of the ligand binding residues. With the results of the single mutants we then generated double and triple mutants, and evaluated the catalytic efficiency towards codeine-6-glucuronide studying the geometry of the docked ligand. Finally, we contrasted the rational mutations designed here with the natural evolutionary pathway of substrate specificity across the Glycosyl Hydrolase 2 family to which this β-glucuronidase belongs.

18) FRUCTOSE-1,6-BISPHOSPHATASE (FBPase) BINDING TO THE VOLTAGE-DEPENDENT ANIONS CHANNEL (VDAC) IS NOT AFFECTED BY SPECIFIC ANTI-FBPase IgG ISOLATED FROM AUTISTIC CHILDREN. .

Asenjo, Joel[1]., Giacaman, Israel[1]., González, Andrea[1]., Vera, Carolina[1]., Francos, Rina[2]., Villarroel-Espindola, Franz[1]., Cuchacovich, Miguel[3]., Concha, Ilona[1]., González-Gronow, Mario[4]., Slebe, Juan[1]., [1]Bioquímica y Microbiología, Ciencias, Universidad Austral De Chile. [2]Psiquiatría Asociación Chilena de Padres de Niños Autistas (ASPAUT). [3]Medicina, Medicina, Universidad De Chile/Hospital Clinico J.J. Aguirre. [4]Ciencias Biomédicas, Medicina, Universidad Católica Del Norte. (Sponsored by FONDECYT 1141033 (JCS); 1130451 (MGG); 3130449 (FVE))

Autism spectrum disorders (ASD) are complex neurodevelopmental syndromes often characterized by clinical deficits in the areas of socialization, verbal and nonverbal communication. Some ASD patients show metabolic alterations as a result of mitochondrial dysfunction. The enzyme fructose-1,6-bisphosphatase (FBPase) reduces the rate of calcium-induced mitochondrial swelling and interacts with mitochondrial proteins involved in membrane permeability. We hypothesize that FBPase could regulate cell mortality/survival and metabolic cell status by its ability to interact directly with the voltage-dependent anion channel (VDAC), a relevant protein on mitochondrial surface. Using ELISA and western blot techniques we found an auto-antibody against FBPase in sera from ASD patients, suggesting that an autoimmune response to FBPase could be a possible cause of the metabolic and physiological changes observed in these patients. Using a modified ELISA system, we demonstrate that liver FBPase interacts directly with VDAC1 *in vitro*, and this association is not affected by isolated anti-FBPase IgG, suggesting that the epitope(s) recognized are not related with the mitochondrial stability modulated by the complex FBPase-VDAC. The role of an auto-antibody against FBPase in autistic patients must be evaluated considering the interaction between FBPase and aldolase and other metabolic enzymes, to understand the role of these autoantibodies in the CNS of ASD patients.

19) Respiratory virus profile in children in Porto Alegre-Brasil during the 2012 post-pandemic period.

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Acute respiratory infections (ARIs) are an important cause of hospitalizations and morbidity in early childhood worldwide. To identify the pattern of circulating viruses, we performed an intensive epidemiological study at the Central Laboratory of Grupo Hospitalar Conceição, one of the largest public health hospitals in the State. Nasopharyngeal samples from 1429 patients <15 years of age who presented signs and symptoms of ARI (seen in the child emergency department), were tested by indirect immunofluorescence (IFI), between January through December 2012. The majority of these children had less than one year of age (79,2%). At least one respiratory virus out of the 7 viruses analyzed was detected in 569 (38,2%) respiratory samples and co-infection of these viruses was detected in 1% (17) of samples. There was clear the variability in seasonal detection of respiratory viruses. The most commonly detected viruses were RSV in 372 samples (26,1%), Parainfluenza-3 in 79 (5,5%) samples and Adenovirus in 46 (3,2%) samples. Forty two children were found with Influenza A (2,9%), of which 39 (93%) was A(H1N1)pdm09 virus. The majority of individuals infected with the A(H1N1)pdm09 strain had 80% presented clinical symptoms (e.g. cough, fever, dyspnea and rhinorrhea). While a large number of ARI infection can be identified as one of the common respiratory viruses in children, approximately 60% of children that present ARI are negative for this diagnostic panel. Thus is it is highly likely that other respiratory viruses co-circulate in Southern Brazil during the season, and therefore this warrants further investigation.

20) Analysis of protein-protein interaction between the cytoplasmic dynein complex light chains and the Murine Leukemia Virus.

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Murine Leukemia Virus (MLV) requieres the breaking of the nuclear envelope during the cell division to reach the host genome. In order to get through the highly dense cytoplasm it probably associates to microtubule associated motor proteins. Indee, we have shown that reduction of the light chains of the cytoplasmic dynein complex Dynlrb2 and Dynlt1, significantly reduces the infection levels of MLV. We do not know if a direct interaction between the cytoplasmic dynein light chains and the viral proteins exists. Here, we analize if a direct interaction between the cytoplasmic dynein complex and the viral proteins capsid is happening in vitro. To do so we purified recombinant GST-tagged Dynlrb2 and Dynlt1 from bacteria and analyzed its interaction with his-tagged MLV CA. Under the assayed condition we do not find a direct interaction between the recombinant proteins. This result suggest that another viral protein, such as p12 or integrase, could be the one interacting with the dynein light chains, also it is possible that the structural features of an infecting virion is what is needed for the interaction. Currently we are testing these two possibilities.

21) High level of genomic diversity of Influenza A viruses associated with severe human disease.

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Influenza A virus, affects mainly the upper respiratory tract and occasionally can cause viral pneumonia. In most of the cases, the severe outcome is related to risk factors (pre-existing comorbidities or high-risk age) but some healthy patients show severity, which have been mainly identified after the H1N1pdm09 outbreak and that remains poorly understood. In this study, serial swabs and blood samples were collected from 82 hospitalized patients diagnosed as positive for influenza A. Comprehensive metanalysis showed that 12% of patients had no risk factors, suggesting that virulence determinants could be mediating disease severity. Unexpectedly, 8 individuals showed a decrease in antibodies titers Influenza A at day 21 post-infection compared to basal antibody titers at day 1, which may be due to antigenic drift of the virus during infection. Viral characterization of isolated strains, subtyped as H1N1pdm09 or H3N2 by qRT-PCR, revealed that H1N1pdm09 strains has high plaque size heterogeneity in culture, and pathogenesis in mice that correlate with the severity of some individuals. Viral genome analysis by Illumina deep-sequencing showed a high diversity and rare mutations, in contrast with H3N2 strains that showed two marked circulating genotypes. Some substitutions, such as HA-S200P, HA-A203T and HA-A214T, might affect the receptor binding site and could contribute to the virulence. We also observed transitional mutations such as NA-I223M, which may show a transient antiviral resistance of these viruses during the treatment. Our results indicate that seasonally, severe influenza infection can be associated with diverse viral genotypes that might harbor yet uncharacterized virulence markers.

22) Wild Bird Influenza Virus Surveillance In Chile

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Avian influenza viruses (AIV) are associated with zoonotic events that can produce animal and human disease, and can potentially generate human epidemics and occasionally pandemics. The main reservoir of AIVs are wild birds. Chile has unique geographical barriers allowing the country to be free from major animal pathogens. However, migratory birds can travel great distances, trespassing these barriers and might introduce pathogens such as AIV. We collected 540 feces and 335 oral/cloacae samples, from wild birds from 5 different locations in continental Chile and Antarctica during the 2014-2015 spring/summer season. 11.5-7.4% of fecal samples and 9.9-27% of oral/cloacae samples were positive for AIV by Matrix qRT-PCR. The positive species identified were: Franklin's gulls (*Leucophaeus pipixcan*), Black Skimmer (*Rinchops niger*), American Oystercatcher (*Haematopus palliates*), Whimbrel (*Numenius phaeopus hudsonicus*), Sanderling (*Calidris alba*), Andean flamingo (*Phoenicoparrus andinus*) and Gentoo (*Pygoscelis papua)* and Shinstrap (*Pygoscelis Antarctica)* penguins. Additionally, 56% (44/78) of serum samples from local wild ducks (*Anas flavirostris*)were found to be positive. We selected qRT-PCR samples with low CT values for amplification and sequencing with the Illumina platform. We obtained complete genome sequences and isolated viruses from three Franklyn gulls corresponding to H13N2 subtypes. These viruses clustered closely with a gull H13N2 AIV found in Chile in 2007, suggesting these AIVs might be endogenous. Our results provide important information of ecological niches that might contribute to the transmission and persistence of influenza viruses in wild birds in Chile, and highlight gulls as potential "carriers" of reassortant viruses from North to South America.

23) Ligand specificity changes produced by cancer mutations

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Recently, several non-synonymous mutations in enzymes had been reported as driver cancer mutations, most notably, mutation R132H in IDH1 and R172K in IDH2, where both change the ligand specificity for cofactor NADPH resulting in the production of an oncometabolite. Our aim is to develop a bioinformatics pipeline to identify ligand specificity changes triggered by cancer mutations in enzymes on a large scale. First, we compare the affinity of the wild-type enzymes and the mutant enzymes with their cognate ligands and with a broad spectrum of chemically similar small molecules. Affinity evaluation is then performed using docking algorithms. Mutant enzyme structures are obtained using the Fold-X algorithm based on the wild-type enzymes; small molecules similar to the cognate ligands are obtained from ligand databases using maximum common substructures as metric. We have validated our methods by processing IDH1 and IDH2 mutations where we rationalized that both mutant IDHs increase their binding affinity for NADPH and decrease their affinity for α-ketoglutarate. These calculations rationalize the kinetic observation that wild type IDHs bind indistinctly NADPH or α-ketoglutarate to then bind CO_2 and proceed to isocitrate, whereas mutant IDHs bind only NADPH which then immediately reduces α-ketoglutarate forming the oncometabolite 2-hydroxyglutarate. We are currently applying this pipeline on a high throughput fashion to significantly mutated enzymes found in over 5000 tumor exomes, to identify whether ligand specificity changes is an isolated observation for IDH or else a common pathophysiological mechanism in cancer etiology. Supported by: Grant Fondecyt 11130578 to DEA.

24) Activation of human platelets by a TLR2 agonist induces aggregation, tissue factor dependent procoagulant activity, thrombin generation and increased adhesion to endothelial cells.

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Introduction: Acute inflammation in response to bacterial infection results in hemostatic abnormalities ranging from subclinical to severe clotting activation, often with devastating consequences. The role of platelets in innate immunity and inflammation is today widely accepted. They express Toll-like receptors (TLR), involved in pathogen recognition and innate immunity activation. Among them, TLR2 is activated by lipoteichoic acid from Gram-positive bacteria and different synthetic molecules. **Aim:** To evaluate whether platelet stimulation with the TLR2-agonist Pam3CSK4, induces aggregation, tissue factor-dependent procoagulant activity (TF-PCA), Thrombin Generation in platelet rich plasma (TG-PRP) and modulate changes on platelet adhesion to intact human pulmonary microvascular endothelial cells (HPMEC). **Methods:** After incubation with Pam3CSK4 or buffer, platelets were evaluated for CD-62, an activation marker by flow cytometry, TF-PCA (FXa generation), TG-PRP (calibrated automated thrombogram) and platelet adhesion to HPMEC (immunostaining). **Results:** Platelet stimulation with Pam3CSK4 induced p-selectin (CD62) exposure of on their surface, and promoted platelet aggregation. This activation caused an enhancement in TF-PCA, compared to unstimulated platelets (p 0.03, n=5) and an augmented TG-PRP (p: at least <0.03, n= 13). Similarly, stimulation of platelet with this TL2-agonist originated oversized aggregates, compared to those generated with a classical coagulation agonist, as TRAP, and promoted greater platelet adhesion to HPMEC than unstimulated platelets. **Conclusions:** Platelet stimulation with TLR2-agonist Pam3CSK4, results in an enhancement of platelet activation and aggregation, TF-PCA, TG and adhesion to endothelium, suggesting that they would play an important role in the prothrombotic state associated with Gram (+) bacterial infections.

25) HSFA2 transcription factor responds to cold acclimation and is a candidate regulator of the dehydrin gene expression in *Eucalytus nitens.*

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During cold acclimation, dehydration may partly contribute by preventing ice formation and ameliorating the effects of freeze-induced cellular dehydration. Extremely hydrophilic proteins such as dehydrins (DHN) are known for its protective role in dehydration. In the context of transcriptional regulation of DHN genes we focused on heat shock factors (HSFs), considering their functions in heat, salt and osmotic stress response. Studies on the relationship of these factors with the *Eni.dhn2* gene would be useful for understanding the regulation of this gene involved in cold acclimation. Heat shock transcription factors (HSFs) specifically bind to *cis*-elements termed heat shock elements (HSEs), which consist of alternating units of the sequence (5'-nGAAn-3'). Through *in silico* analyses we identified the sequence 5'-TAAATTTTTT-3' in the *Eni.dhn2* promoter as an HSF binding site. The relative expression of gene *Eni.dhn2* increased after exposure to cold temperatures and reached a maximum level after exposure to freezing temperatures, as observed by RT-qPCR. The same results were observed for the *Eni.hsfa2.* This increment after cold and freezing conditions could be related to a decrease in free water, as a consequence of cold acclimation. By performing EMSA assays using nuclear extracts isolated from leaves of plants of *E.nitens* exposed to cold temperatures, we tested binding activity to five putative transcription factor binding sites present in the *Eni.dhn2* promoter, observing a specific interaction only to a probe containing the HSFA2 binding site identified in this promoter. This interaction was not observed in the absence of treatment.

26) Zebrafish as an infection model of *Flavobacterium psychrophilum*.

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F. psychrophilum an opportunistic pathogen responsible for the Bacterial Cold Water Disease (BCWD) and the rainbow trout fry syndrome (RTFS), which mainly affects fish from *Salmonidae* family causing significant mortality in salmonid aquaculture, featured by skin ulcers and damaged in caudal fin tissue. Nowadays, there is not a suitable animal model to study *F. psychrophilum* infection. Currently, zebrafish has risen as a model to try pathogen infection interesting to aquaculture. In this study we assay if *F. psychrophilum* is able to infect zebrafish larvae.

Zebrafish larvae at 48 hour post-fertilization (hpf) were exposed by immersion with 10^8 UFC/ mL of *F. psychrophilum* in E3/TYES medium and monitored one day post-infection (1dpi). We evaluated larvae phenotype, bacteria presence, and activation of neutrophils using *Tg(mpx:GFP)* larvae. To evaluate the effect of innate immune response reduction, we did knockdown of Pu.1 by a specific morpholino.

We found similar phenotypes in muscle and fin described for rainbow trout infected with *F. psychrophilum*. Also, we detected activation of neutrophils, indicating activation of the innate immune response on infected larvae. The reduction of neutrophil number by Pu.1 knockdown increased the amount of infected larvae.

Muscle and caudal fin phenotype observed in zebrafish larvae presented a distinctive clinical sign described on *F. psychrophilum* infected rainbow trout. These results indicate that bath infection is a suitable method for study the infection by *F. psychrophilum* in zebrafish.

27) Anticancer effect of Ru(II) on gastric (AGS) cancer cell line

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Introduction: In Chile, gastric cancer (GC) is the third leading cause of death with 3,371 cases in 2012. This high occurrence has led to developing of new therapies based on inorganic compounds, which have better anticancer activity and minor side effects. Between these, transition metals such as ruthenium have shown great.

In this work we showed the anticancer activity of a $[(Pdbi^{-2})Ru^{2+}(etpy)]^{0}$ complex on AGS gastric cancer cells.

Methods: AGS cells were grown on 96 well plates and ruthenium was added to concentrations of 1, 5, 10, 15, 25, 50, 75 and 100mM respectively. The cellular viability was determined by the MTT method, and the IC50 was estimated. For other hand, the expression of the pro-apoptotic genes p53, Bax and caspase-3 was evaluated by qPCR in the AGS cells treated with 25 and 75mM of Ru(II).

Results: We observed a notorious decreased of cellular viability to concentrations of ruthenium of 50, 75 and 100 µM. The IC50 for our complex of Ru(II) was 25,11µM. The expression of caspase-3 was higher in both treatment regarding the control, but the expression of p53 was higher only with 25µM of ruthenium respect of control. Interestingly the expression of Bax was down-regulated in both treatments.

Conclusions:Our results are interesting, because we have synthesized a new drug based on ruthenium(II) with antiproliferative properties, capable to alter the expression of pro apoptotic genes, which could be an interesting opportunity for the treatment of gastric cancer.

28) Identification of an efficient molecular marker for diagnosis of causal agent of walnut blight by Loop-Mediated Isothermal Amplification (LAMP)

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The Gram-negative bacterium *Xanthomonas arboricola pv. juglandis* (*Xaj*) is the etiologic agent of walnut blight in *Juglans regia*, a disease that affect walnut production world-wide. The disease produce necrotic leaf, dark brown spots on fruits, exudates and blighted and branches. In Chile, in 1982 was the first *Xaj* outbreak report and now the Bío-Bío region is affected strongly. Although exist a constant monitoring of the disease, is absolutely necessary the development of an efficient and field-applicable diagnosis system. In this work was applied and optimized an innovative PCR-derived technique, named Loop-mediated Isothermal Amplification (LAMP) for *Xaj* diagnostic.

In this work, were selected eleven putative genetic markers as target for LAMP amplification. Finally, once the best molecular marker was selected by experimental procedures, all amplification conditions were optimized (temperature, time and reagent concentration). Additionally, the specificity of the best primer set was tested against different field isolates. All DNA samples were provided by AgroAdvance Laboratory Ltda. and Servicio Agricola y Ganadero de Chile (SAG).

Our results show that *hpaA* gene was the best genetic marker for LAMP amplification. The designed primers were highly specific against *Xaj*, not showing amplification with other *Xanthomonas* isolates (3 *X. campestri* and 1 *Xhantomonas sp*.) or *Pantoea* isolates. Finally, the amplification conditions were standardized, with an optimal temperature of 63ºC and 30 minutes of reaction.

In summary, our results show LAMP as a promising alternative for a rapid, efficient and field-applicable *Xaj* diagnosis in Chile.

***29) Clostridium difficile spores* binds C1q and C3 complement proteins and contributes to entry into intestinal epithelial cells.**

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Clostridium difficile is a Gram-positive, anaerobic, spore-former, which has become the main cause of diarrhea associated with antibiotics. Episodes of *C. difficile*-associated infections are successfully treated with antibiotics. However recurrence rates caused by the remaining spores reaching up to 60% and the mechanism involved in the persistent of the spores in the host are still unknow. C. difficile spores can adhere to epithelial cells and also it was previously shown that inactivation of complement system decreases this adherence. In this study we explored interactions between *C. difficile* spores, intestinal epithelial cells, also with the complement proteins C1q and C3. Here, we report the internalization of *C. difficile* into intestinal epithelial cell line Caco-2 and their decrease in absence of C1q and C3 in a dose-dependent way. In vitro experiments show that spores can recruit C1q and C3 from human serum in a dose-dependent way. Also the recruit of C1q and C3 occurs in different clinical isolates and is species-specific. The recruit of C1q and C3 by the spore was decrease in absence of the surface spore protein BclA which one is a collagen-like protein same as C1q. 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.

30) Knockdown of the mitochondrial antisense ncRNAs abolishes murine melanoma tumor growth and metastasis

Burzio, Verónica[1,2]., Lobos-Gonzalez, Lorena[1]., Silva, Verónica[1]., Araya, Mariela[1]., Oliveira-Cruz, Luciana[1].,Fitzpatrick, Christopher[1,2]., Briones, Macarena[1]., Villegas, Jaime[1,2]., Villota, Claudio[1,2]., Vidaurre, Soledad[1,3]., Borgna, Vincenzo[1]., Lopez, Constanza[1]., Teresa, Socías[1]., Valenzuela, Sebastian[4]., Restovic, Franko[1]., Echenique, Javiera[1]., Burzio, Luis[1,2]., [1]Cancer Lab Andes Biotechnologies/Fundacion Ciencia & Vida. [2]Ciencias Biológicas Universidad Andrés Bello. [3]Ciencias Químicas y Biológicas Universidad Bernardo O`higgins.[4]Bioterio Fundacion Ciencia & Vida. (Sponsored by FONDECYT 1110835/1140345)

The mitochondrial non-coding RNAs (ncmtRNAs) are differentially expressed in normal and tumor human cells. The sense transcript (SncmtRNA) is expressed in normal proliferating and tumor cells. The antisense transcripts (ASncmtRNAs) are also expressed in normal proliferating cells, but not in resting cells. Remarkably, ASncmtRNAs are down-regulated in all tumor cells (cell lines and cancer biopsies) studied so far. Knock-down of the ASncmtRNAs induces apoptotic death of several human tumor cell lines, but not normal cells. Analogously, this family of transcripts also exists in mouse cells and expression patterns of these transcripts in normal and tumor murine cells are identical to human. Knock-down of the ASncmtRNAs markedly inhibits murine melanoma B16F10 cell proliferation and induces apoptosis, potentiated by down-regulation of survivin, as in human cells. *In vivo* studies in a syngeneic B16F10 melanoma model revealed a reduction in survivin levels in primary tumors, confirming the antisense effect of the treatment. Most remarkably, the treatment induced a complete inhibition of tumor relapse and metastasis, after surgical resection of primary tumors. These pre-clinical results represent a new discovery on the relationship between lncRNAs and cancer and strongly suggest that the ASncmtRNAs are potent targets to develop a safe and effective therapeutic approach for melanoma, a highly invasive form of cancer.

31) Angiotensin-(1-9) reverses stress-induced autophagy in cardiomyocytes

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Introduction. Angiotensin-(1-9) is a component of the Renin-Angiotensin-System (RAS) whose biological effects counteracts the cardiovascular actions of Ang II (hypertension, cardiac hypertrophy, vascular remodeling). Autophagy, the recycle system for cellular components, has a great impact on cardiac cells biology: either increments or repression of autophagy beyond a narrow range have been related to cardiac pathologies, including hypertension and stroke. Here, we asked if Ang-(1-9) modulates autophagy in cardiomyocytes induced by nutrient deprivation or Ang II.

Methodology. Primary cardiomyocytes from rat pups were cultured in DMEM/M199 (4:1) medium supplemented with 2% FBS. 100 µM Ang-(1-9) was added to cells previously treated with Ang II or incubated in RPMI medium. Autophagic flux was evaluated in cells incubated with cloroquine (30 µM) by 3 hours previous to the end of the stimuli. Autophagy was evaluated by western blot, monitoring the protein levels of LC3-II, Beclin1 and GAPDH.

Results. Our results show that Ang-(1-9) modestly but effectively inhibited autophagy in cardiomyocytes after 6 hours of incubation. When cells were previously treated with Ang II or RPMI, autophagy was induced, and the presence of Ang-(1-9) restore the autophagy to baseline level.

Conclusions. Ang-(1-9) is a natural peptide with proved anti-hypertensive, anti-hypertrophic and anti-remodeling activity. The results presented here showing Ang-(1-9) as a inhibitor of stress-induced autophagy gives some light on the mechanisms by which Ang-(1-9) exerts its protective actions. Thus, Ang-(1-9) represents a potential pharmaceutical target for autophagy-related diseases.

32) Study of the expression of Galectin-3 in an *in vitro* model of smooth muscle cells derived of pulmonary artery under hypoxia

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Introduction.Pulmonary arterial hypertension is caused by vasoconstriction of pulmonary arteries, thrombosis *in situ* and vascular remodeling. During the latter process, hypoxia induces an antiapoptotic phenotype and increase proliferation of vascular smooth muscle cells. Galectin-3 (Gal-3), a β-galactoside-binding protein with antiapoptotic function, has been shown to be up-regulated under hypoxic conditions. Here, we tested if Gal-3 expression is induced by hypoxia in SMCs derived from cultured human pulmonary artery (hPASMC) and if it modulates hPASMC survival and apoptosis.

Methodology.Cultured hPASMC were exposed to hypoxia for 24 hours. After stimulation, cycle cell and cell proliferation analyses were performed. The proteins levels of HIF-1α and Gal-3 were evaluated by Western blot, while the mRNA of Gal-3 were evaluated by RT-qPCR. The subcellular localization of Gal-3 was assessed by immunofluorescence.

Results.hPASMC express Gal-3 at baseline. Under hypoxic conditions, mRNA level of Gal-3 increases by 50%. In contrast the protein levels falls to a 30%. In parallel, the subcellular localization of residual Gal-3 switched from nuclear (normoxic) to a broad intracellular localization (hypoxic). **Conclusions**. In spite that our first guess about Gal-3 expression under hypoxia was not fulfill, the data showed here is encouraging considering that one Gal-3 function on cytosol is to inhibit apoptosis. To modify the activity of Gal-3 either genetically or pharmacologically is still pertinent and necessary to establish a potential role for Gal-3 on vascular remodeling.

33) The folding mechanism of an artificial knotted protein characterized by optical tweezers

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Protein folding occurs because establishment of week interactions observed in the native state (native contacts) predominate over the formation of transient interactions that could promote alternative conformations (non-native contacts). However, knotted proteins defies this view because their polypeptide chain needs to form non-native contacts during its folding. To determine the role of non-native contacts in such topologies, we explored the folding mechanism of an artificial knotted protein (2ouf-knot). Hence, optimization of non-native contacts should play a minor role during its folding. The folding mechanism of 2ouf-knot was characterized maintaining the knotted topology in the unfolded state by pulling their C and N extremes using optical tweezers. The molecular stretching of 2ouf-knot obtained at constant velocity showed a single transition of folding/unfolding. Using the worm like chain formalism, we calculate an experimental contour length that was 30 % shorter than expected. However, observation of molecular fluctuation at constant force showed a hidden intermediate between the native and unfolded state under equilibrium conditions. In this regime of mechanical stress, the overall molecular extension observed between the native and unfolded state agrees with the theoretical length of 2ouf-knot. These results contrast with the absence of intermediates in the case of a natural knotted protein (MJ0366) characterized under the same experimental setup. We suggest that the intermediate observed in the case of artificial knot arise by the lack of specific non-native contacts, that otherwise will help to smooth the energetic landscape of natural knotted protein.

34) In silico discovery of novel TRPV1 agonists

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The TRPV1 channel is a polymodal non-selective cation channel that integrates diverse stimuli in the pain generation pathway. TRPV1 is known to be activated by ligands like capsaicin or by noxious heat (>42°C) but the structural events that the channel undergoes during gating remain unknown. The TRPV1 structure was solved by cryo-electron microscopy (Cryo-EM), providing information about the vanilloid binding site but unfortunately the binding mode of the agonist could not be observed. Due to the paradoxical analgesic effects of TPRV1 this has become the subject of a number of studies and the characterization of the agonist binding provides exciting opportunities for pharmacological intervention of the channel. We have analyzed the vanilloid binding site in order to develop a model for the structure-function relationship of capsaicin. By implementing a computational strategy, we have identified a population of compounds that potentially could interact with TRPV1 based on their predicted binding affinity, octanol-water partition coefficient and the ability for satisfying the binding mode of capsaicin. These compounds were experimentally tested using the Two Electrode Voltage Clamp (TEVC) in *XenopusLaevis* oocytes expressing TRPV1 channels. In well agreement with our in silico predictions the compounds show binding affinities in the nanomolar range giving us valuable insights to propose new candidates compounds for the development of pain relief therapies.

35) Molecular Modeling, docking and MM-GBSA calculations in predicting the binding mode and biding free energies of dihydropyridazinones derivatives as PDE3A inhibitors

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Phosphodiesterases (PDEs) are enzymes that catalyze the hydrolysis of the 3'-5' phosphodiester bond of cyclic nucleotides (cAMP and cGMP) [1], therefore providing a major pathway for modulating cyclic nucleotide signaling. Changes in the intracellular concentration of these messengers produce an inhibition of platelet aggregation [2]. As a result, PDEs are established as novel targets for prevention and treatment of cerebral ischemia/thrombotic disorders and cardiovascular diseases. In this work, we performed a computational structure-based study to predict the binding modes of around one hundred antiplatelet agents that are known as PDE3A inhibitors. In this sense, we built a homology model of the catalytic portion of PDE3A using the crystallographic structure of PDE3B (PDBid: 1SO2) and subsequently molecular docking study was performed in order to predict the binding mode of the PDE3A inhibitors. The docking poses were used for the estimation of relative free energy biding through Molecular Mechanic Generalized Born Surface Area (MM-GBSA) method and provided good statistical correlations (Q^2 = 0,763) between the experimental and theoretical biding data (pIC_{50}, $\Delta\Delta G_{bind}$, respectively). Our *in silico* study provides a new tool for predicting the affinity of dihydropyridazinones derivatives. It can be used for guiding the design and synthesis of novel, selective and more potent PDE3A inhibitors. References 1. Gresele, P., Momi, S., & Falcinelli, E. (2011). Anti-platelet therapy: phosphodiesterase inhibitors. *British Journal of Clinical Pharmacology*, *72*(4), 634–646. 2. Boswell-Smith, V., Spina, D., & Page, C. P. (2006). Phosphodiesterase inhibitors. *British Journal of Pharmacology*, *147 Suppl* , S252–S257.

36) Cloning, heterologous expression and purification of Laccase α from *Trametes versicolor*

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Laccases are multicopper oxidases of great biotechnological interest because of their utility in various industrial applications. The high redox potential of the copper atom in their active site allow them to oxidize a wide variety of aromatic substrates. How do the laccases active site residues tune the copper redox potential, is still an open question. In this work we setup an expression and purification system for the *Trametes versicolor* laccase α, which will be used in further studies to understand the determinants of the redox potential in laccases. The gen AY693776 that encodes the Laccase α from *T. versicolor*, was cloned in a modified pYES2 plasmid which confers geneticin resistance as a selection marker. For heterologous expression, the S288c *Sacharomyces cerevisiae* strain was transformed with this plasmid and expression was induced at 30°C in SC-Galactose medium. Laccase activity in culture supernatants was spectrophotometrically evaluated at different induction timepoints and purification steps, by measuring the production of oxidized substrate ABTS at 420nm. After three days of induction, the supernatant showed specific activity of 0.04 U/mg in 60 µg of total protein from a 50 ml culture. The optimization of the expression conditions involved the evaluation of different copper concentrations, the effect of biotin, different carbon sources and phosphate-buffered pHs. The higher yields were obtained at 20°C in a buffered SC-medium at pH 5.6. After concentration by stirred ultrafiltration, the culture supernatant was purified in an hydrophobic interaction chromatography column, resulting in a 10-fold increase of the specific activity.

37) The exosporium morphogenetic proteins, CdeC and CdeM, are essential for the assembly and morphogenesis of the outermost exosporium-like layer of *Clostridium difficile* spores.

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Clostridium difficile is a major nosocomial pathogen that has become a major cause of antibiotic-associated diarrhea. *C. difficile* spores have an important role in the pathogenesis of *C. difficile* contributing to infection, persistence and transmission. Evidence has demonstrated that *C. difficile* spores have an outermost layer, termed the exosporium, which is involved in host-spore interactions which seem to be relevant to the initiation of the infection and for recurrent infections. However, the mechanism of the exosporium assembly remains unclear. In this study, we characterized the functional role of CdeC and CdeM in the spore´s ultrastructural and functional phenotypes. Through the construction of independent isogenic knockout mutants in the *cdeC* and c*deM* genes of the *C. difficile* 630 strain, we showed that: (i) while CdeC is required for correct assembly, CdeM is essential for the formation of exosporium layer; (ii) *cdeC* and *cdeM* spores were more sensitive to lysozyme, ethanol, and heat treatment than wild-type spores; (iii) Absence of CdeC and CdeM affects the recruitment of other exosporium proteins such as CotA, CotB, BclA1, BclA2, BclA3, CdeA and CdeB; (iv) absence of the exosporium layer in cdeM spores is associated with higher levels of adherence to Caco-2 cells compared to wild-type spores. In conclusion, these results have characterized two morphogenetic proteins, CdeC and CdeM, which are involved in the assembly of the spore´s outermost layer.

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38) The exosporium morphogenetic proteins, CdeC and CdeM, are essential for the assembly and morphogenesis of the outermost exosporium-like layer of *Clostridium difficile* spores.

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Clostridium difficile is a major nosocomial pathogen that has become a major cause of antibiotic-associated diarrhea. *C. difficile* spores have an important role in the pathogenesis of *C. difficile* contributing to infection, persistence and transmission. Evidence has demonstrated that *C. difficile* spores have an outermost layer, termed the exosporium, which is involved in host-spore interactions which seem to be relevant to the initiation of the infection and for recurrent infections. However, the mechanism of the exosporium assembly remains unclear. In this study, we characterized the functional role of CdeC and CdeM in the spore´s ultrastructural and functional phenotypes. Through the construction of independent isogenic knockout mutants in the *cdeC* and c*deM* genes of the *C. difficile* 630 strain, we showed that: (i) while CdeC is required for correct assembly, CdeM is essential for the formation of exosporium layer; (ii) *cdeC* and *cdeM* spores were more sensitive to lysozyme, ethanol, and heat treatment than wild-type spores; (iii) Absence of CdeC and CdeM affects the recruitment of other exosporium proteins such as CotA, CotB, BclA1, BclA2, BclA3, CdeA and CdeB; (iv) absence of the exosporium layer in cdeM spores is associated with higher levels of adherence to Caco-2 cells compared to wild-type spores. In conclusion, these results have characterized two morphogenetic proteins, CdeC and CdeM, which are involved in the assembly of the spore´s outermost layer.

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39) Caveolin-1 expression in metastatic breast cancer cells promotes exosome formation

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Introduction Breast cancer is one of the cancer malignancies with the highest mortality rates in females in Chile, although the use of screening programs and the application of adjuvant therapy have lead to improvements in this respect. Despite such advances, the development of metastasis remains the cause of over 80% of deaths associated with this and other cancers. Caveolin-1 (CAV1), a multifunctional membrane protein that is upregulated in final stages of cancer disease, promotes migration and invasiveness of tumor cells, raising the question as to how CAV1 contributes to this behavior of cancer cells. More recently exosomes, small approximately 100 nm vesicles that transport proteins and micro-RNAs, have been implicated in metastasis. Thus, an intriguing possibility in this respect was that CAV1 expression may enhance exosome release from tumor cells to the immediate or distant environment where they would serve to promote metastasis by mechanisms that remain to be defined.

Methodology Ultracentrifugation (UC) and filtration methods were used to purify exosomes from supernatants of metastatic MDA-MB-231 wild-type(wt), MDA-MB-231(shCAV1) (possessing the plasmid pLKO.1 encoding a “small hairpin” directed against CAV1, as well as MDA-MB-231(shControl) and non-metastatic T47-D human breast cancer cells. Subsequently, exosomes were quantified by ELISA and the BCA method. Microparticle size was determined by Dynamic Light Scattering (DLS) and transmission electron microscopy (TEM). The presence or absence of CAV1 and the exosome marker CD63 was detected by Western blotting.

Results Analysis by DLS and TEM revealed the presence of microvesicles ranging from 50 and 120nm in size when prepared by the UC method and between 50 to 300nm when using the filtration method. MDA-MB-231(wt) and shControl derived exosomes also contained CAV1 and CD63. Low levels of CAV1 were detected in exosomes from MDA-MB-231(shCAV1) compared to (wt) cells and no CAV1 was detected exosomes from the T47-D cell line. Notably greater amounts of exosomes were isolated from supernatants of MD-MB-231(wt) and (shControl) cells compared to MDA-MB-132(shCAV1) and T47-D cells.

Conclusions The UC method was identified as being the most suitable approach to purify exosomes given that the observed microparticle size was in the range expected based on the literature. Importantly, CAV1 expression appears to promote exosome release from metastatic breast cancer cells.

40) Mitotic Bookmarking of mmp genes by Ski co-repressor.

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During mitosis, chromosome condensation is coupled to a general inhibition of gene expression. A fundamental biological question is how genes expression patterns are retained during mitosis and sustained after cell division, e.i., at early G1?. Ski is a transcriptional co-repressor that recruits HDAC-dependent machinery to a variety of transcription factors, including SMADs. In mouse embryonic fibroblasts (MEFs), Ski localizes as discrete pair of dots at the pericentromeric region of a number of condensed mitotic chromosomes. Previously, we identified pericentromeric genes that were repressed at early G1 in a Ski-dependent manner, indicating that the presence of Ski was required for silencing of certain genes immediately after cell division. Most repressed genes were *mmp*3, *mmp*10 and *mmp*13 which are located in a cluster at the pericentromeric region of chromosome 9. By chromatin immuno-precipitation assays (ChIP), we found that Ski occupies *mmp's* promoter at the SMAD binding element. Ski occupies *mmps* gene promoters, in these regions Ski absence results a 4-fold loss of H3K9me3 mark that is replaced by a ~5-fold increasing in K9 acetylation during mitosis. Finally, we showed that the absence of Ski increased cell mobility and invasion, which was related with an increased MMPs activity demonstrated by zymograms experiments. These results suggest that Ski has an active role supporting fibroblast specific gene program and retaining phenotype during cell division. FONDECYT:1151435.

41) Ski protein localization in nucleolus and NOR regions in human cells

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Ski is a transcriptional co-repressor that acts as a negative regulator of gene expression in a variety of pathways, including TGF-β. Ski expression varies through cell cycle reaching the highest levels during mitosis. At this stage, Ski is required for proper chromosome segregation in mouse fibroblasts, although the underlying mechanism has not been identified yet. Here, we studied the distribution of Ski in mitotic human cells, including primary and non-transformed fibroblasts (MRC5), as well as breast epithelial tumor cell line (MCF7). By Indirect Immunofluorescence, we found that Ski is located in centromeric and/or pericentromeric regions of acrocentric chromosomes. As most of these chromosomes in human have NORs (Nucleoli Organization Regions), next we assessed whether Ski associates with the transcriptional machinery of ribosomal genes (rDNA). By indirect co-immunofluorescence in non-synchronized cells and in metaphase plates, we determined the colocalization of Ski and transcriptional factor for rDNA, UBF, in nucleoli and NORs, respectively. We found that in interphase nuclei, Ski co-localized with UBF at nucleoli, with a distinct distribution pattern depending on the cell types. On the other hand, in mitosis Ski co-localized with UBF in acrocentric chromosomes, however localization of Ski on those chromosomes was not depending on UBF. Finally, chromatin immunoprecipitation experiments showed that both Ski and UBF occupied the promoter of ribosomal genes. These results suggest a role for Ski on transcriptional regulation of ribosomal genes by associating with NOR regions during mitosis to maintain epigenetic information in these regions. FONDECYT 1151435

42) GLP-1 prevents mitochondrial and phenotypic changes induced by PDGF-BB in VSMC through PKA-Drp1 pathway.

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Vascular smooth muscle cells (VSMC) change from a contractile to a proliferative/migratory phenotype during vascular pathologies. We propose that mitochondrial dynamics play a key role during platelet-derived growth factor-BB (PDGF-BB)-induced VSMC dedifferentiation. In this work, we evaluated the modification of mitochondrial dynamics induced by GLP-1, an incretin used for type 2 diabetes treatment, on VSMC dedifferentiation. VSMC A7r5 were pre-treated with GLP-1 (100 nM) for 3 h and then with PDGF-BB (10 nM) for 0-24 h. GLP-1 prevented PDGF-BB-induced mitochondrial fragmentation, as visualized by confocal microscopy using Mitotracker Orange. GLP-1 inhibited mitochondrial fragmentation by inducing Drp1 phosphorylation through a PKA-dependent mechanism. GLP-1 also blocked PDGF-BB-dependent mitochondrial potential decreased, measured by JC-1 and flow cytometry. PDGF-BB increased A7r5 proliferation, determined by [^{3}H]-thymidine incorporation, and migration, determined by wound healing and transwell assays. PDGF-BB-induced A7r5 dedifferentiation was inhibited by GLP-1 pretreatment. Our results suggest that GLP-1 prevents PDGF-induced VSMC dedifferentiation by regulating mitochondrial dynamics and function. Thus, the use of GLP-1 analogs during type 2 diabetes treatment could modulate mitochondrial dynamics producing positive effects on vascular physiology.

43) Glucagon-like peptide 1 (GLP-1) inhibits VSMC dedifferentiation through an autophagy-dependent mechanism

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Vascular smooth muscle cells (VSMC) respond to changes in the local environment by adjusting their phenotype from contractile to migratory. Platelet-derived growth factor BB (PDGF-BB) is a potent inductor of this phenotypic switching and plays a key role in vascular diseases, including atherosclerosis. We propose that transformation from contractile to migratory phenotype requires protein degradation by autophagy. Furthermore, glucagon-like peptide-1 (GLP-1), an incretin used for treating type 2 diabetes, prevents atherosclerosis development in diabetic patients. Whether GLP-1 inhibits PDGF-BB-dependent phenotypic switching in VSMC by regulating autophagy remains unexplored. VSMC A7r5 were treated with GLP-1 (100 nM), PDGF-BB (10 nM) or GLP-1 + PDGF-BB. Phenotypic switching was assessed by wound healing assay and contractile protein levels. Autophagy was assessed by LC3 II, LC3 I and p62 protein levels. Our results showed GLP-1 prevented PDGF-BB-induced cell migration at 24 h. Furthermore, GLP-1 inhibited the decrease on contractile proteins induced by PDGF-BB at 24-48 h. PDGF-BB induced autophagy at 24-48 h. Pretreatment with GLP-1 inhibited the PDGF-BB-dependent autophagy activation. Inhibition of autophagy with chloroquine inhibited PDGF-BB-induced phenotypic switching. These findings suggest that autophagy inhibition by GLP-1 could be part of the mechanism used by GLP-1 to inhibit PDGF-BB-induced VSMC dedifferentiation.

44) Deletion of hinge loop residues allows domain swapping of the cold shock protein from *Bacillus caldolyticus*.

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The cold shock protein of *Bacillus caldolyticus* (*Bc*CSP) is a small 7 KDa protein composed of two subdomains, corresponding to 2 and 3 β-strands connected through a hinge loop that folds into a β-barrel. Recently, a crystallographic structure of *Bc*CSP in the presence of hexathymidine was solved as a domain swapped (DS) dimer, where the two-stranded subdomain is exchanged with the adjacent subunit. However, molecular size-exclusion chromatography (SEC) of *Bc*CSP with DNA showed a unique monomeric form. In other DS proteins, such as p13suc1 and cyanovirin, it has been observed that increased flexibility and opening of the hinge loop has a major role. Therefore, we explored the effect of site-specific deletions of hinge loop residues E36 or G37 (*Bc*CSPΔ36 or *Bc*CSPΔ37) and double mutant *Bc*CSPΔ36-37, in allowing DS in *Bc*CSP. The single mutants did not lead to DS even at 2 mM protein concentration. Nevertheless, the double mutant *Bc*CSPΔ36-37 has two populations that correspond to dimeric and monomeric forms, as ascertained by SEC. These species are interchangeable and its dissociation constant at 37ºC is in the mM range. Moreover, these mutants show reversible folding by temperature with melting temperatures of 76.0, 79.4, 82.2 and 76.6°C and ΔG_u of 9.3, 9.6, 10.2 and 8.6 Kcal•mol^{-1} for *Bc*CSPwt, *Bc*CSPΔ36, *Bc*CSPΔ37 and monomeric *Bc*CSPΔ36-37, respectively. Consequently, the deletions on the *Bc*CSP hinge loop did not significantly decrease protein stability. Our results suggest that DS of *Bc*CSP involves dissociation of the subdomains and opening of the hinge region without stability loss.

45) SERINE 294 IN THE HUMAN GLUT1 TRANSPORTER IS CRITICAL FOR ACTIVITY

Castillo, Boris[1]., Hidalgo, Sofia[1]., Lagos, Ingrid[1]., Perez, Alejandra[1]., Ojeda, Lorena[1]., Cuevas, Alexei[1]., Salas, Monica[1]., Reyes, Alejandro[1]., [1]Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral De Chile. (Sponsored by Supported By FONDECYT 1130386, FONDEF D11I1131)

The glucose transporter GLUT1 catalyzes the facilitative diffusion of glucose into erythrocytes and handles glucose supply to the brain and other organs. The structure of the crystal of the GLUT1 transporter has been recently described at 3.2 Å resolution (Deng et al. *Nature 510*, 121-125, 2014). This structure suggests that the residue S294 form part of the external lid that obliterates the cavity that bound glucose from the external surface of the protein, when the carrier stays in the inward-facing conformation. To probe this assertion, we design the mutant S294A and express it into Xenopus oocytes. Here, we report the functional properties of this construct. Using 2-DOG as substrate, the S294A isoform retains a similar K_M value compared with the hGLUT1 wild-type, but the V_{max} decreased dramatically. Besides, Western blotting showed that the mutant have expression levels that were comparable to those of wild-type. These observations are compatible with the notion that Serine 294 plays a critical role in the mechanism that close the translocation channel, separating the sugar binding cavity from extracellular water during substrate translocation.

46) **Identification of the pathway of *Clostridium difficile* spore-entry into intestinal epithelial cells.**

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(Sponsored by Proyecto FONDECYT Regular 1151025)

Clostridium difficile is a major nosocomial pathogen that has become a major cause of antibiotic-associated diarrhea. *C. difficile* spores play an essential role in the pathogenesis of *C. difficile* contributing to infection, persistence and transmission. *C. difficile* spores are impermeable to antibiotics and the have been suggested to persist in the colonic tract. However, the mechanism(s) through which *C. difficile* spores persist in the colonic tract remain unclear. In this work, we demonstrate by transmission electron microscopy that *C. difficile* spores are able to invade intestinal epithelial cells (IECs). Using pharmacological inhibitors, we demonstrate that *C. difficile* spores internalize into IECs through a mechanism that involves clathrin, caveolin and macropynosomes. Importantly, we demonstrate by confocal fluorescence microscopy, that *C. difficile* spores localize with clathrin, caveolin and macropynosomes. Collectively, these results provide evidence of a novel mechanism of persistence of *C. difficile* in the host.

47) Structural Determinants of Dinucleotide Specificity in Genetically Encoded Fluorescent Sensor *Peredox*

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In the Nicotinamide adenine dinucleotides, the presence of a phosphate group at the adenine ribose gives them functionality divergence, since NADP(H) is principally involved in anabolic processes and NAD(H) into catabolic processes. The genetically encoded fluorescent sensor *Peredox* posseses a Rossmann fold domain, from the transcriptional repressor T-Rex, that is specific for NAD(H) and allows the *in vivo* measurements of NADH/NAD ratio. In order to determine which residues would exert a major influence on dinucleotide specificity, a structure-evolution analysis and statistical potential calculation were performed on T-Rex. Our structural analysis showed that the Rossmann fold domain from T-Rex is structurally related to members of the 6PGDH family (average RMSD of 2.65 Å). This family contains both NAD-binding and NADP-binding Rossmann fold domains with well-defined sequence motifs located in a loop region equivalent to β4-β5 loop in T-Rex. This analysis suggests that the D112N-V113R-D114S mutation would favor NADP-specificity in T-Rex. The statistical potentials, a tool that transform protein-cofactor interactions frequencies of structures in the PDB to binding free energies , reinforce the idea that D112 plays a key role in NAD-binding. An *in silico* arginine-scanning suggests that V113R mutation would improve NADP-binding This analysis will be used to design a variant of *Peredox,* through site-directed mutagenesis, which can perform *in vivo* measurements of NADPH/NADP ratio

48) Effect of copper on the interaction of *Arabidopsis thaliana* with the metal resistant bacterium *Cupriavidus metallidurans* CH34.

Clavero, Claudia[1]., Ruiz, Daniela[1]., González, Bernardo[1]., [1]Laboratorio de Bioingeniería, Center for Applied Ecology and Sustainability, Universidad Adolfo Ibáñez. (Sponsored by FONDECYT 1151130 Y CAPES FB 0002-2014)

Plants exude a complex mixture of compounds and constitutes an intricate network of signals for rhizosphere microorganisms. Composition of plant roots exudate varies both by biotic and abiotic factors (e.g. soil composition). Copper is one of the more important metals in Chilean soils; In small quantities acts as a micronutrient to plants and their associated microorganisms; however, at high concentrations it produces a toxic effect. It's unknown how copper stress affects the composition of plant exudates, and variations on the exudates composition may affect differentially the rhizosphere microorganisms. On the other hand, soil bacteria such as *Cupriavidus metallidurans* CH34 can tolerate high concentrations of copper and other metals, and their potential effect on these kind of bacteria on plant growth under normal or copper stress conditions is poorly understood. In this study, the overall composition of copper stressed plant root exudates and how this stress influences both the plant and the bacterial growth, will be analyzed using *Arabidopsis thaliana* and *C. metallidurans* interaction as study model. In presence of the bacteria, significant effects had been observed in plant roots exposed to copper and a distinctive composition of exudate compared to normal conditions.

49) Expression of carotenogenic genes in mutant strains for two general transcriptional repressors in the yeast *Xanthophyllomyces dendrorhous*.

Córdova, P[1]., Alcaíno, J[1]., Baeza, M[1]., Cifuentes, V[1]., [1]Laboratoio de Genética, Ciencias Ecológicas, Facultad de Ciencias, Universidad De Chile. (Sponsored by Fondecyt 1140504. CONICYT Doctoral Fellowship)

Introduction

Xanthophyllomyces dendrorhous is a carotenogenic yeast producer of astaxanthin, pigment of commercial interest due to its use in aquaculture and pharmaceutical industries. Currently, it is known the carotenoid biosynthetic pathway and the expression profile of carotenogenic genes in this yeast. However, regulatory mechanisms of this process are still unknown. It has been demonstrated that glucose exerts a repressive effect on carotenogenesis in *X. dendrorhous* by down-regulating the corresponding genes at a transcriptional level. Possible regulatory transcriptional factors involved in catabolite repression like *MIG1*, *GTR1* and *GTR2* were identified and genetically characterized in our laboratory. The aim of this study was to determine the effect of the *GTR1*and *GTR2* mutations as these genes encode general transcription repressors possible involved in glucose mediated suppression of carotenogenesis.

Methodology

By using mutant strains *gtr1*$^{-}$and *gtr2*$^{-}$, it was evaluated the effect of this mutations on carotenoid production by extraction and spectrophotometric quantitation of total pigments produced. The expression of genes involved in carotenogenesis and related pathways was assessedby quantitative RT-PCR on cDNA obtained from total RNA isolated from yeast cultures grown in presence or absence of glucose.

Results

Carotenoid synthesis is not suppressed in both mutant strains in presence of glucose as it is in the wild-type strain. At transcriptional level, mutant strains showed a lower repression of some carotenogenic genes mostly from those involved in early steps of the pathway.

Conclusions

The *GTR1* and *GRT2* genes of *X. dendrorhous* are functional and partially involved in regulation of the carotenogenic process.

50) CHEMICAL MODIFICATION REVEALS THAT THE HUMAN GLUT2 CARRIER HAS A EXOFACIALLY ACCESSIBLE THIOL GROUP IMPORTANT FOR ACTIVITY

Cuevas, Alexei[1]., Lagos, Ingrid[1]., Toledo, Matias[1]., Arce, Robinson[1]., Castillo, Boris[1]., Ojeda, Lorena[1]., Perez, Alejandra[1]., Salas, Monica[1]., Reyes, Alejandro[1]., [1]Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral De Chile. (Sponsored by Supported 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. The GLUT2 protein has six cysteine residues, and we decide to probe the functional role of these residues by using thiol-alkylating reagents. When the wild-type GLUT2 expressed into Xenopus oocytes was incubated with either MTSET, a thiol-alkylating membrane-impermeant reagent, or N-ethylmaleimide, a freely membrane permeant thiol reagent, activity was decreased by around 50%. Therefore, we designed a cysteine-less isoform of the GLUT2 carrier (C-less GLUT2) to obtain a transporter refractory to thiol reagents. The properties of the C-less GLUT2 expressed into Xenopus oocytes were similar to those of the native GLUT2, except that activity was lower than of the wild-type transporter and similar to that of the alkylated wild-type GLUT2. These data suggests that at least one exofacial thiol group is important for sugar transport activity.

51) Expression analysis of exogenous genes involved in plant growth promoting effects in neutral plant associated bacteria *Cupriavidus Pinatubonensis* JMP134.

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The plant growth-promoting rhizobacteria, known as PGPR, have different action mechanisms to produce a visible beneficial effect on the growth of different plant species, many which are of commercial interest. Among these mechanisms, the synthesis of 1-Aminocyclopropane-1-carboxylate (ACC) deaminase allows to reduce the ethylene levels in plant, preventing some of the adverse effects that this hormone generates over plant development. Moreover, Indoleacetic acid (IAA) synthesis promotes plant cell elongation which implies an increase in root length and number of lateral roots. The expression of these genes is conditioned by several biotic and abiotic factors in the rhizosphere, whereby a better regulation of their expression could improve the plant-bacteria benefic association. The purpose of this study was to clone genes involved in plant growth promotion into *Cupriavidus Pinatubonensis* JMP134, which is able to colonize the roots steadily but possess not characterized mechanisms to promote plant growth. For this purpose, *acdS* and IAA tryptophan-dependent synthesis genes (*iaaM* and *iaaH*) were inserted separately into the pBS1 plasmid, which possess an inducible promoter that is able to control the expression of the inserted genes. Once pBS1:Acds and pBS1:IAA vectors were obtained they were used to evaluate their expression in JMP134 strain and to assess their effect upon plant growth. The results suggest that exogenous expression of these genes can affect different plant growth parameters but not at the same level as described in PGPR. Better regulation of their expression could improve or match the efficiency observed in other PGPR species.

52) Synthesis and *in silico* analysis of the quantitative structure–activity relationship of heteroaryl-acrylonitriles as AChE inhibitors

De La Torre, Pedro[1]., Treuer, Adriana V.[2]., Gutierrez, Margarita[2]., Poblete, Horacio[3]., Alzate-Morales, Jans H.[1]., Trilleras, Jorge[4]., Caballero, Julio[5]., [1]Centro de Bioinformática y Simulación Molecular, Facultad de Ingeniería, Universidad De Talca. [2]Instituto de Química de los Recursos Naturales Universidad De Talca. [3]Institute of Computational Comparative Medicine, Department of Anatomy and Physiology, Universidad de Kansas. [4]Departamento de Ciencias Básicas Universidad del Atlantico. [5]Centro de Bioinformática y Simulación Molecular, Departamento de Ingeniería, Universidad De Talca. (Sponsored by PD Thanks The Doctoral Program Of Applied Sciences At Univer- Sidad De Talca, As Well As The Chilean International Cooperation Agency (AGCI) And CONICYT-Chile For A Doctoral Fellowship (Folio Beca 63130202). MG Thank FONDECYT Project 110048)

Alzheimer disease (AD) is a neurodegenerative disorder that causes damages in brain due to factors like oxidative stress, low-levels of the neurotransmitter acetylcholine, tau and β-amyloid protein aggregation[1-3]. Given the increase of people suffering from AD, it is necessary to explore new compounds as a new alternative for designing of novel drugs for the AD treatment. Recently, the heteroaryl-acrylonitriles have emerged as a new family of acetylcholinesterase inhibitors (AChEIs)[4-5]. A study of the structure-activity relationship of these compounds could help to elucidate the main molecular features that contribute to the activity of these compounds. In this work, we performed 3D-QSAR analyses through a Comparative Similarity Indices Analysis (CoMSIA) to determine the key-factors for the activity of *E/Z*-heteroarylacrylonitriles reported in literature and novel derivatives that are reported in this work for the first time. The compounds were synthetized via Ultrasound and Microwave-Assisted Knoevenagel reaction[6]. The biological activities as AChEIs were explored and the compounds exhibited EC_{50} values on a micromolar (μM) scale. The best CoMSIA model included both electrostatic and hydrogen bond donorfields (CoMSIA-ED model) and provided a highest Q^2 value of 0.901. Our *in silico* study provides a new tool for predicting the affinity of heteroaryl-acrylonitriles as AChEIs and it can be used for guiding the design and synthesis of novel, selective and more potent AChEIs. **[1]** *Neurochem. Res.* 1998;23:135–40. **[2]** *Neuron* 1996;16:881–91. **[3]** *Trends. Neurosci*. 1993; 16:460–5. **[4] De-la-Torre *et al*.** *Molecules* 2012; 17:12072–85. **[5]** Parveen *el al*, *New. J. Chem.* 2014; 38:1655–67. **[6] De-la-Torre *et al*.** *Ultrason. Sonochem.* 2014; 21:1666–74.

53) THOR-O: An Optogenetic-Oscillatory system in *Saccharomyces cerevisiae*

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A living cell is a highly organized system. Currently, traditional approaches dissect the network under study within its basic parts. But thanks to synthetic biology, it is possible “to create for understanding” taking components from natural networks and rearranging them to gain insights into the logic of the networks. One of the most paradigmatic transcriptional networks correspond to oscillators. Transcription-based oscillators have caught attention because they control relevant processes such as cell cycle and circadian rhythms. Recently, there has been engineering of these types of feedback loops in living cells and the construction of these oscillators provides an example of how the interplay between the analysis of naturally occurring systems, mathematical modelling and the construction of synthetic circuits can increase the basic knowledge of the phenomenon under study. Through a bottom-up approach, we are planning to uncover and test the underlying molecular mechanisms governing the basic principles of TTFL (Transcriptional-Translational Feedback Loop) architecture system using the photo adaptation components of *N. crassa* in *S. cerevisiae*. This proposed synthetic biology circuit has been named herein THOR-O (Transcriptional Hybrid Optogenetic Regulated Oscillator). The basic architecture of the THOR-O system is going to be similar to the Neurosporamolecular clock, which is composed on a time-delayed negative feedback loop where the positive module activates the expression of a negative module that will repress its own activator. This network topology is also similar to the architecture of the *N. crassa* photo adaptation system, but the main contrast compared to the pacemaker architecture is that it does not oscillate but instead leads to attenuation response (producing photo adaptation). As part of the design of the TTFL, we are using two proteins from the *N. crassa* photo adaptation in which the output of this system is a protein that can compete the above mentioned interaction, inhibiting by transcriptional light-switch and inhibiting therefore its own expression. This process creates a negative feedback loop with a time-delay, which will depend on the kinetics of transcription, translation, nuclear degradation and so on. These parameters will allow us to challenge the THOR-O system with variations in the dynamics of the negative and positive modules, driving it to oscillatory properties and providing insights regarding TTFLs.

54) Analysis of extracytoplasmic function sigma factors in the plant growth-promoting rhizobacteria *Burkholderia phytofirmans* PsJN

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Nutrient availability makes plant internal tissues and rhizosphere attractive spaces for microbial colonization. However, bacteria interacting with plants need to adapt to constant environmental changes, reacting to fluctuations in their immediate surroundings through global gene regulation. Sigma factors are dissociable subunits of RNA polymerase that regulate gene transcription initiation by recognition of specific promoter sequences. Their total number in the genome of a specific bacterial strain is usually determined both by its lifestyle and its genomic size. The extracytoplasmic function sigma factors (ECFsf) are the largest and more diverse group of these factors (classified into 43 subgroups), but little is known about their specific functions, although they are predominant in environmental and plant associated bacteria, which makes them interesting candidates for global gene regulation players during plant colonization. The aim of this study was to address the role of ECFsf present in the plant growth-promoting rhizobacteria *Burkholderia phytofirmans* PsJN. This strain encodes numerous ECFsf, which were inactivated by insertional mutagenesis. Results suggested that at least one ECFsf is related with growth and general metabolic processes in strain PsJN, meanwhile the other ECFsf may be specifically involved in oxidative stress tolerance, biofilm formation and siderophore biosynthesis, all of them processes related to plant colonization by bacteria. These results support a possible role of ECFsf in plant bacteria interactions, at the level of the rhizosphere.

55) Web platform of citizen science: experience with a collaborative project on image analysis for the evaluation of a novel treatment against tumor cell proliferation.

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Citizen science can be defined as a collaborative effort between citizens and scientists in order to carry out a certain task. During the last few years, developed countries have implemented several platforms of citizen science with the purpose of informing and integrating citizens into the scientific realm. In Chile, there is still a very low participation level of citizens in the research of our scientists. With this in mind, the citizen science foundation advises and develops collaborative scientific projects with citizens, with the intention of generating communication bridges between national research and the community. Thus, we developed the first web platform of citizen participation along with the group of the researcher Luis Burzio, who investigates mitochondrial RNA markers (ncmtRNAs) which differentiate cancer cells from normal cells. Through fluorescent micrographs, users (>200 people), registered on the platform, participated in the identification and quantification (with the computer mouse) of tumor cells after the novel antineoplastic treatment under development in this research group. Afterwards, the system delivered the final number of cells counted by users, which was used by the responsible researcher. Our results are promising due to the reduction in time consumption for the counting process and an increase in statistic robustness.

Fundación Ciencia Ciudadana, Fondecyt 1140345

56) *twist* and *cxcr4* are regulated by Hif-1α during the neural crest development

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Hypoxia induced-factor 1 (HIF-1) is the master regulator of the hypoxic (low oxygen tension) response. Under this condition HIF-1 is stabilized, favoring the regulation of hypoxia-related target genes. Studies on human tumor cell lines showed that HIF-1 regulates TWIST and CXCR4 by direct binding to their promoter regions. Our laboratory has demonstrated that Hif-1α plays an essential role on neural crest cells migration during embryo development. However, the *in vivo* mechanism by which Hif-1α regulates *twist* and *cxcr4* has not been elucidated. Zebrafish embryoswere treated with $CoCl_2$, a Hif-1α stabilizer. Then gene expression for *twist1a* and *cxcr4a* was analyzedby *in situ* hybridization and qPCR. Bioinformatic analysis was performed to detect potential hypoxia response elements (HREs) on each promoter. Those potential HRE were tested by Chromatin Immune Precipitation (ChIP) to analyze the enrichment of Hif-1α on the selected promoter regions. Finally the functionality of each HRE was evaluated by luciferase assay. By *in silico* analysis we founded seven and six putative HREs on the promoters of *twist1a* and *cxcr4a*, respectively. Our results from ChIP and luciferase assay indicate that both genes, *twist1a* and *cxcr4a*, are regulated by direct binding of Hif-1α *in vivo*. Demonstration of *in vivo* activation on gene expression by Hif-1 on *twist1a* and *cxcr4a* genes, will help us to understand the complex gene regulation during neural crest cells migration.

57) mRNA-seq analysis reveals liver fibrosis in response to handling stress in the red cusk-eel (Genypterus chilensis)

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Introduction: The red cusk-eel (*Genypterus chilensis*)has been considered one of the marine species of greatest farming potential in Chile. Nevertheless, the culturing of this species is seriously hindered by scarce biological knowledge and, primarily, limited information on the negative effects of stress associated with intensive farming in marine species. **Material and Methods:** Total RNA was extracted from liver 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. RNA-seq analyses were validated through RT-qPCR. **Results:** We identified *in silico* 262 up-regulated and 298 down-regulated genes. Gene ontology enrichment analysis revealed a significant up-regulation of genes associated to liver angiogenesis and down-regulation of genes associated to steroids metabolism. These results were validated by RT-qPCR analysis of candidate genes. **Discussion:** Our results indicate that handling stress up-regulated expression gene of important fibrosis signaling pathways in *G. chilensis*. This study contributes to 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

58) Evaluation of inflammatory parameters of foliar extracts of two genotypes from murtilla (*Ugni molinae* Turcz.) in cardiac fibroblasts

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Introduction: Cardiac pathologies occur with an overproduction of pro inflammatory mediators. Cardiac fibroblasts (CF) measure inflammatory environments and produces extracellular matrix proteins for tissue reparation. CF's role is quite relevant in fibrosis and inflammation associated pathologies. *Ugni molinae* is a Chilean native bush. Leaf extracts have proven *in vivo* analgesic and anti-inflammatory properties, allocated to pentacyclic triterpenoids and phenolic compounds. Genotypic variety within the species is responsible for different chemical profiles from the leaves extracts. **Objective**: To evaluate the effect of two different genotypes leaves extract, EET22-1 (ethanolic extract) and EAE23-2 (ethyl acetate extract) on inflammatory mediators. **Methodology**. Extracts were prepared from dried minced leaves from two genotypes with growing polarity solvents. Fibroblast culture was prepared from neonate rats, and kept in DMEM/F-12, FBS 10%. Cells were stimulated with 1µg/mL LPS and the extracts (0,1-1-10 y 100 µg/mL) for 24 hours. Cell viability was measured by MTT assay, protein levels of p65 and IL-1β by western blot and MMP-9 by zymography. **Results**. EAE23-2 (100 µg/mL) decreased cellular viability to 20%, and EET22-1 decreased it to 36% with 500 µg/mL, in relation to the untreated control. There were no significant decrease between the pre and post treated groups in relation to the LPS control in terms of p65 phosphorylation, IL-1β levels and MMP-9 activity. **Conclusions**. EAE23-2 showed to be more toxic than EET22-1, probably for its higher concentrations of triterpenoid compounds. The extracts did not reverted or prevented the inflammatory response induced by LPS. More experiences are necessary for MMP-9 measurement.

59) The Non-Coding mitochondrial RNAs are new players in the regulation of the Cell Cycle of human cells

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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 with chemically modified oligonucleotides, in mouse and human cancer cell lines, induces massive apoptotic death, without affecting viability of normal cells. Cell death is preceded by a marked proliferative arrest at the G1/S checkpoint, characterized by a strong dowregulation of cyclins D1 and B1, which is not reflected in changes in the mRNA levels of these proteins. We hypothesized that these effects could be explained by a mechanism involving putative new mitochondrial microRNAs (Mito-miRs) generated from the ASncmtRNA. miR-1973 has been proposed as one of these miRNAs and, in accordance with our hypothesis, its expression is upregulated after oligonucleotide-induced knockdown of the ASncmtRNAs. Interestingly, expression of the mature miR-1973 and the ASncmtRNA2, show an inverse correlation throughout the cell cycle in the A375 melanoma cell line. We propose the ASncmtRNAs as novel players in cell cycle regulation and hypothesize that they could constitute a novel source of miRNAs.

60) Computational flexible backbone design of peptide binding sequences for the TEV protease

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The Tobacco etch virus (TEV) 3C-type protease is an enzyme widely used in biotechnological applications. It has a stringent substrate specificity given by strict interactions, that accommodate the reacting motif into an extended beta strand conformation, mimicking an intrinsic part of the protease\'s structure. Alternative binding peptides, which could help into broaden or facilitate its biotechnological applications have not yet been reported. Therefore, designing new substrates and/or inhibitory sequences, with high affinity, is an interesting, yet difficult task. Here, we propose a rational approach to design new peptide sequences that could bind the active site of TEV protease. A flexible backbone design methodology was applied to uncover new models with broad backbone conformations. Structures were selected based on interface energy of the complex and structural and sequence cluster analysis. Molecular dynamics and free energy methods were applied to evaluate the selected models regarding the consensus substrate. Results suggest that models could bind the active site in a variety of conformational poses. Also, a correlation between the relevant catalytic distance and energy interface was found, indicating that favorable-energy models have a substrate-like conformation, imposing a difficulty to design good inhibitory sequences with our approach. Nevertheless, these results gave us insight for improvements of our current design methodology.

61) *In silico* characterization of proteins implicated in the core of the exportation channel of the Dot/Icm secretion system in *Piscirickettsia salmonis*

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P. salmonis is a Gram-negative bacterium, pathogen of different salmonid species, that impact negatively the chilean salmon industry. Recent reports have confirmed the presence of a Dot/Icm secretion system in *P. salmonis*. The function of this system has been widely characterized in *Legionella pneumophila* and *Coxiella burnetii*, showing being implicated in all aspect related with intracellular multiplication.

This work was focused in modeling the structural proteins of the exportation channel of the Dot/Icm system in *P. salmonis*, in order understand its structure and their putative interaction with the exported virulence effector.

For channel reconstruction was use as model the *L. pneumophila* Dot/Icm apparatus. To do so, was made a screening in the *P. salmonis* genome using the *L. pneumophila* DotC, DotH, DotD, DotG and DotF proteins in the RAST server. The proteins with the higher match score were modeling using the RaptorX, Phyre2 and IntFOLDS servers. All modeling images were processed with the MacPymol and VMD Softwares.

Were detected 5 proteins encoded in *P. salmonis* genome, homologues to *L. pneumophila* DotC-H-D-G-F. The predicted structural model of *P. salmonis* proteins is conserved with those described for *L. pneumophila*. Additionally, was possible determine by qPCR that this proteins are overexpressed at earlier times of infection *in vitro*, coinciding with the bacterial establishment in vacuolar compartment inside infected cells.

Finally, our results are consistent, showing a highly structural conservation among *P. salmonis* and *L. pneumophila* Dot/Icm machinery. qPCR results shows a correlation between *dot/icm* gene expression and bacterial internalization and intracellular multiplication.

62) Studying the Binding Affinity Determinants of a Derivative Series from Sulbactam and Clavulanate as β-Lactamase Inhibitors.

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β-Lactamases are responsible for the hydrolysis of the β-lactam ring on many known antibiotics, thus conferring resistance to bacteria. The increase in the number of β-lactamases and other resistance mechanisms, together with the few new antibiotics discovered or approved in the past three decades, leaves us in an alarming situation for overcoming the antibiotics resistance problem (Draws 2010, Worthington 2013). The use of β-Lactamase inhibitors, in conjunction with antibiotics, is a common treatment in clinical ambient but microorganisms had also become resistant to former. There are a few clinically used inhibitors: Clavulanate, Sulbactam and Tazobactam. Most of the research has been focus into solve the resistance problem doing the synthesis and biological testing of new antibiotics. On the other hand, Nottingham *et al* (Nottingham 2011) and Turnowsky *et al* (Turnowsky 1983) synthetized some sulfonamide like sulbactam series and clavulanate analogs. Our aim is to understand the structural and thermodynamic determinants in the affinity of those reported Sulbactam and Clavulanate β-Lactamases inhibitors. To do so, molecular dynamic simulations (MDS), docking, covalent docking and MMGBSA (Alzate-Morales 2014) calculations are employed. MDS results so far, showed that some specific residues like SER130 and Arg244 established strong and durable interactions with the inhibitors. Both of them are implicated in resistance. Free binding energy and biological data correlations are not conclusive in this series and some improvements in the MMGBSA protocol and scoring function will be implemented.

63) The RNA helicase DDX3 connects CRM1-dependent nuclear export and translation of the HIV-1 unspliced RNA

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DEAD-box RNA helicase DDX3 is a host factor essential for HIV-1 replication and thus, a potential target for novel therapies aimed to overcome viral resistance. Previous studies have shown that DDX3 promotes nuclear export and translation of the HIV-1 unspliced mRNA. Although the function of DDX3 during both processes requires its catalytic activity, it is unknown whether other domains surrounding the helicase 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 the regulation of HIV-1 unspliced mRNA translation initiation. Our results suggest that the intrinsically disordered N-terminal domain of DDX3 regulates its functions in translation acting prior the recruitment of the 43S pre-initiation complex onto the viral 5´-UTR. Interestingly, this regulation was dependent on the CRM1-dependent nuclear export pathway suggesting a role of the RNA helicase in interconnecting nuclear export with translation of the HIV-1 unspliced mRNA.

64) Reduced level of tetrahydrobiopterin and phosphorylation associates with lower nitric oxide synthase activity in HUVECs from maternal supraphysiological hypercholesterolemia

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Maternal physiological hypercholesterolemia (MPH) occurs in pregnancy assuring fetal development; however, maternal supraphysiological hypercholesterolemia (MSPH) leads to endothelial dysfunction and aortic atherosclerosis in fetal vessels. Normal endothelial function requires nitric oxide synthase (NOS) activity, a phenomenon regulated at least by Akt– and PKC–dependent activatory (P-Ser^{1177}) andinhibitory (P-Thr^{495}) phosphorylations, as well as by the cofactor tetrahydrobiopterin (BH_4). **Aim**: To determine the effect of MSPH in NOS activity in human umbilical vein endothelial cells (HUVECs). **Methods and Results**: MSPH was determined considering a cut-point >280 mg/dL for maternal total cholesterol at term of pregnancy (n=30). In HUVECs from MSPH, NOS (L-citrulline formation from L-arginine) activity was reduced (81 ± 10%) compared with MPH. The NOS protein abundance was unaltered, but P-Ser^{1177}– and P-Thr^{495} –NOS/total NOS ratio were reduced (40 ± 5 and 25 ± 5%, respectively) compared with MPH. The P-Akt/total Akt ratio was reduced (40 ± 7%) in MSPH, without changes in PKC protein abundance.The level of BH_4 was lower (78 ± 8%) compared with MPH The mRNA expression, protein abundance and activity of GTPCH1 (i.e., enzyme for BH_4 synthesis) were also reduced (85± 8%, 57 ± 8% and 83 ± 7%, respectively) in MSPH.Interestingly, sepiapterin (100 µM, 24 hours) restored the BH_4 and NO levels in cells from MSPH to comparable values in MPH . **Conclusion:** MSPH associates with reduced NOS activity due to reduced NOS phosphorylation and BH_4 synthesis. This mechanism could be involved in endothelial dysfunction and later development of atherosclerosis described for MSPH offspring.

65) Targeted next-generation sequencing (NGS) of *MICA gene* in Gastric Cancer

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Gastric cancer (GC) is one of the leading causes of mortality in most Asian and South American countries. Its current incidence in Chile is 30 per 100,000 habitants. MICA is a natural killer (NK) group 2D (NKG2D) ligand, whose interaction with NKG2D receptor triggers NK cell-mediated cytotoxicity toward target cells, and is a key molecule in tumor immune surveillance. Several lines of evidence indicate that single-nucleotide polymorphisms (SNP) of the *MICA gene* have been associated with tumor immune evasion. In this study, we performed targeted resequencing of *MICA* exons in GC and investigated the presence of mutations in this gene. Genomic DNA was isolated from tumor tissue samples of 50 patients with gastric adenocarcinoma who underwent gastrectomy. Targeted sequencing with Illumina MiSeq TruSeq Custom Amplicon (TSCA; 12 amplicons, 500x coverage) was then carried out. All research was performed in agreement with institutional ethical committees guidelines and approval. Sixty-two single nucleotide variants (SNV) were found in the *MICA gene*. 39 variants were found in more than one patient and 11 of these variants were present in a Chilean database of SNPs. Interestingly, 3 of these variants have not been described previously. Our data demonstrate the feasibility of using NGS to identify genomic variants in the *MICA gene*. However, further analyses are necessary to define the effect of these gene variants in the protein structure and function, as well as their role as a prognostic or predictive marker in GC.

66) IMPACT OF THE ADENOSINE METHYLATION MACHINERY ON HUMAN IMMUNODEFICENCY VIRUS GENE EXPRESSION

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N6-methyladenosine (m6A) is a reversible post-transcriptional modification in eukaryotic mRNAs with an important impact on gene expression. The effects of this modification are regulated by three classes of proteins that have been classified as writers, erasers, and readers of m6A. The writer complex is involved in methylation at the consensus sequence RRACH and is composed by methyltransferases METTL3 and METTL14 together with the RNA-protein WTAP. The erasers correspond to the m6A RNA demethylases ALKBH5 and FTO, which specifically remove the methyl group from adenosines making this process reversible. The reader proteins are responsible of detecting and interpret the m6A modification in mRNA and correspond mainly to members of the YTH-domain family such as YTHDF2.

In addition to cellular mRNAs, m6A modifications have been found in some viral transcripts suggesting a control of gene expression during replication cycle. Data from transcriptome-wide bioinformatic analyses revealed that the methylation consensus sequence RRACH is significantly more frequent in the HIV-1 unspliced mRNA compared to cellular mRNA, suggesting that HIV-1 could modulate its gene expression using the m6A modifications.

To study m6A impact on HIV gene expression, we overexpressed m6A writers, erasers and readers complexes analyzed their impact on protein synthesis from an HIV-1 reporter provirus.

Our preliminary results show that overexpression of m6A writers and readers has a positive impact on protein synthesis from the HIV-1 unspliced mRNA suggesting that viral gene expression can benefit from this host mRNA modification.

67) Innate immune responses associated with severe influenza A virus infection in humans.

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The presence of single nucleotide polymorphisms (SNPs) in genes of the innate immune system, might affect the ability to mount an adequate response in influenza A virus infection, resulting in aberrant responses and severe disease. Interleukin 28B (IL28B) is a potent antiviral cytokine and its expression level depends on the presence of SNPs rs12979860 (CT/TT/CC) and rs8099917 (TG/GG/TT). While the TT and GG haplotype relate to basal levels, the C and T alleles in each case are associated with increased IL28B expression. We evaluated by qRT-PCR these SNPs in the IL28B gene of 92 individuals infected with Influenza A, and determined seroconvertion by hemagluttination inhibition assay, assessed pro- and anti-inflammatory cytokines profiles of 18 cytokines using a Multiplex ELISA, and analyzed clinical metadata of individuals to determine the relation between these factors and disease severity and outcome. Preliminary results indicate that non-severe patients only have the IL28B genotypes related with higher expression levels, whereas severe individuals can have the basal level genotypes. Additionally, non-severe individuals also had higher seroconversion rates, as compared to the severe group and both groups appear to have similar cytokine expression profiles, where cytokines IL-10 and 12 are expressed robustly, and IL-8, 13, IFNg and MIP1a are have reduced expression. Therefore, a severe influenza could be inversely associated with the expression of IL28B. These results expand our understanding of host factors contributing to the pathogenesis of this disease, which can have an impact on therapeutic and prophylactic interventions targeting those at higher risk severe disease.

68) Growth/Differentiation Factor 11 (GDF-11) restores energy metabolism in hypertrophic cardiomyocytes

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GDF-11 belongs to the TGF-β superfamily and has been involved in cardiac tissue differentiation during embryonic development. However, GDF-11 also reverses age-related cardiac hypertrophy (CH) and *in vitro* prevent cardiomyocyte hypertrophy induced by catecholamines. CH is a maladaptive process that increases functional heart demand and lead to functional, morphological and metabolic remodeling of cardiomyocytes. A hallmark of cardiac hypertrophy is a change of mitochondrial metabolism, which lead to changes in energy substrate utilization, dysfunction of the electron transport chain and decreased ATP synthesis capacity, resulting in an overall deterioration of the cardiac metabolic function. We hypothesized that GDF-11 restores the energy metabolism dysfunction in cultured hypertrophic cardiomyocytes. To test this, primary cultures of neonatal rat cardiomyocytes were treated with norepinephrine (10 µM for 48 h) in the presence or absence of recombinant GDF-11 (10 nM). Cardiomyocyte hypertrophy markerswere assessed by epifluorescence microscopy analysis. Energy metabolism was determined by measuring intracellular ATP levels and by Seahorse extracellular flux analyzer. Our results showed that GDF-11 prevents the increase of hypertrophy markers induced by NE, showing its antihypertrophic action. NE decreases intracellular ATP levels and largely increases oxygen consumption rate in cultured cardiomyocytes, indicating a failure in energy metabolism. Combination of NE and GDF-11, as well as GDF-11 itself, increase intracellular ATP levels and the maximal mitochondrial respiration capacity in cultured cardiomyocytes, indicating that GDF-11 enhanced mitochondrial function and prevent the energy metabolism decline induced by NE in cultured cardiomyocytes. These results suggest that GDF-11 prevents pathological CH by increasing mitochondrial energy metabolism.

69) Polycystin-1 regulatesIGF-1-induced cardiomyocyte hypertrophy and IGF-1 receptor signaling

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Cardiac hypertrophy is an early cellular process triggered by chronic biomechanical stress and growth factors such as insulin-like growth factor 1 (IGF-1). The molecular mechanisms involved in the development of cardiac hypertrophy remains uncertain. The binding of IGF-1 to its receptor (IGF-1R) stimulates its own phosphorylation and activation of Akt and ERK signaling cascades. Polycystin-1 is a plasma membrane protein found in different cell types, including cardiomyocytes. In kidney cells, polycystin-1 acts a mechanosensor. Recently our group showed a similar role for this protein in mechanically stressed cardiomyocytes (Pedrozo et al. Circulation, 2015).

In this work we hypothesize that polycystin-1 regulates IGF1-induced hypertrophy. Our results show that knock down of polycystin-1 protein reduces IGF1-dependent cardiomyocyte hypertrophy assessed by β-myosin heavy chain (β-MHC) protein levels and morphometric parameters such as cell perimeter, area and sarcomerization degree. The phosphorylation of molecular targets of IGF1R signaling pathways such as IGF-1 receptor, ERK and Akt were attenuated in polycystin-1 knock down cardiomyocytes, without changes in protein levels of IGF-1R. The mechanism involves the reticulum membrane protein tyrosine phosphatase 1B. In summary our results suggest that polycystin-1 mediatesIGF-1-induced cardiomyocyte hypertrophyand regulates IGF-1R signaling pathway

70) Effects of distance variations between a positioned nucleosome and a DNA regulatory element on nucleosome remodeling dynamics.

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Chromatin dynamics is fundamental for gene regulation in eukaryotic genomes and ATP-dependent remodeling complexes play an important role in it. The yeast SWI/SNF complex is the first characterized remodeling complex and has several remodeling activities; among them is nucleosome displacement in *trans* (nucleosome eviction), which is connected to transcription initiation in several genes from yeast to humans. We have previously shown that this activity is enhanced in SWI/SNF when recruited to a nucleosome by a transcription factor. The present work is in the context of understanding the effects that small insertions and deletions occurring at gene promoters can have on the catalytic activity of SWI/SNF by altering the position and/or the rotational phase taken by the complex on DNA respect to a positioned nucleosome after recruitment by a transcription factor. With this purpose we carried out remodeling assays using different mononucleosomes reconstituted *in vitro*, which vary in the number of base pairs between a positioned histone octamer and a binding site for the transcription factor Gal4. We tested 15 different mononucleosomes having an “octamer to binding site” distance ranging from 8 to 22 bp. Our remodeling assays were visualized through electrophoretic analysis on non-denaturant gels. We did not observe differences in the binding activity of the transcription factor Gal4-VP16 to the different probes. However, when testing SWI/SNF nucleosome eviction activity under recruitment by Gal4-VP16, we observed distance ranges with maximal and minimal nucleosome eviction. Our results point to a new mechanism linked to chromatin dynamics.

71) Development of a coupled Deacetylase-Demethylase enzymatic assay

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Epigenetic regulation of gene expression involves the active participation of histone modifying enzymes. Among this group of proteins, there are Histone Deacetylases and Demethylases which remove acetyl and methyl groups from the histone tails, leading to transcriptional repression and/or chromatin condensation. Our research group studies the histone deacetylase-demethylase complex LHC, constituted by the Lysine-specific demethylase 1 (LSD1), the histone deacetylases HDAC1/2 and the transcriptional co-repressor CoREST. For the biochemical characterization of our recombinant HDAC1 and HDAC2 containing-complexes, we have developed a novel enzymatic assay that enables the measurement of the deacetylase activity on a biological relevant substrate. This assay takes advantage of both the functional association between HDAC1/2 and LSD1, and the catalytic properties of LSD1, whose specific demethylase activity on di- and mono-methylated Lys4 of histone H3 is abolished by hyper-acetylation of the substrate. By testing a family of histone H3 peptides, methylated on Lys4 and acetylated on different Lys residues, we have selected the suitable substrate for an assay in which H3 tail deacetylation is monitored by measuring the LSD1-catalysed demethylation of Lys4. To further validate the measurement procedure of our deacetylase assay, we determined the steady-state kinetic parameters of HDAC1 and HDAC2 recombinant complexes and the inhibition constant (K_i) for Suberanilohydroxamic acid (SAHA), by performing competitive inhibition assays. The obtained results were no different from those previously published with different experimental methods, demonstrating that the HDAC-LSD1 coupled assay is an efficient and reliable tool for catalytic analysis of HDAC1 and HDAC2 isoenzymes

72) MEF31 is a PPR protein necessary for editing of the *orfX* mitochondrial transcript in *Arabidopsis thaliana*

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RNA editing is a post-transcriptional modification occurring in plant cloroplasts and mitochondria. It consists in C to U changes that are necessary for obtaining mature mRNAs encoding functional proteins. PPR (pentatricopeptide) proteins are nuclear-encoded factors giving specificity to this process by recognizing a nearby region just upstream of the specific C to be edited. Here, we describe the characterization of AT2G46050 as Mitochondrial Editing Factor 31 (MEF31), a PPR protein that is necessary for specific editing at site 581 of the *orfX* transcript in *Arabidopsis thaliana*. Confocal microscopy experiments with stably transformed plants showed that the 100 N-terminal amino acids of MEF31 are able to target GFP to mitochondria. A massive analysis of editing at 269 sites in *A. thaliana* by the SnaPshot method showed a defect in editing at the *orfX*-581 position in a homozygous mutant line for MEF31 (*mef31-1*). RT-PCR and cDNA clones analysis confirmed editing abolition at *orfX*-581 in this and in a second mutant (*mef31-2*), and additionally detected a decrease in editing rate at the nearby *orfX*-586 site. Recognition of *orfX*-581 site by MEF31 is consistent with the recently proposed code for target recognition by PPR proteins. The defect in editing at the 586 position is silent, but the change at the 581 position modifies a highly conserved amino acid in the ORFX protein, which is homolog to the *E. coli* TatC protein. However, *mef31* mutants and wild type plants showed no differences in development when grown under standard long day conditions. Other conditions must be tested to elucidate the importance of this conserved amino acid in ORFX function in plant mitochondria.

73) Cloning of two LIMCH1 isoforms: characterization of their distribution in rat brain and their agmatinase activity.

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Agmatine, a precursor for polyamine biosynthesis, is also associated with neurotransmitter, anticonvulsant, antineurotoxic and antidepressant actions in the brain. This molecule results from the decarboxylation of L-arginine by arginine decarboxylase, and it is hydrolyzed to urea and putrescine by agmatinase. Recently, we have described a new protein that also hydrolyzes agmatine, agmatinase-like protein (ALP), which was identified through immunohistochemical analysis in the hypothalamus and hippocampus of rats. However, its sequence differs greatly from all known agmatinases and does not contain the typical Mn^{2+} ligands associated with the urea hydrolase family of proteins. ALP has a LIM-like domain close to its carboxyl terminus, and its removal results in a truncated variant with a 10-fold increased kcat value and a 3-fold decreased Km value for agmatine. Analysis of the gene database revealed several transcripts, denominated LIMCH1 isoforms, with extreme 3´ sequences identical to ALP. Limch1 gene products have been described as members of a multi-domain family of proteins with the biggest isoform containing a calponin homology (CH) domain at its N-terminus. We cloned two LIMCH1 transcripts, one of 3177 bp and the other of 2709 bp (ALP contains 1569 bp) and analyzed LIMCH1 expression and distribution in rat brain using RT-PCR, Western blot and immunohistochemical analyses. LIMCH1 was detected mainly in the hypothalamic and hippocampal regions, which is similar to the distribution of ALP and agmatine in brain. In addition, we cloned and expressed both isoforms in *E. coli*, and confirmed that they were catalytically active on agmatine with kinetic parameters similar to ALP. LIM domain-truncated variants of both isoforms moderately increased the kcat and catalytic efficiency. Thus, we propose that LIMCH1 is useful to regulate the intracellular concentrations of the neurotransmitter/neuromodulator, agmatine. VRID-Enlace Universidad de Concepción 215.037.019-1.0.

74) THERMODYNAMIC STUDY OF THE INTERACTION BETWEEN THE PROTEIN PKA AND MUTANTS OF KEMPTIDE CONTAINING HOMOARGININE

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Protein kinases (PKs) are enzymes that modify the function of other proteins by attaching phosphate groups to them. They constitute a large and diverse family of enzymes that play critical roles in multiple signaling networks in eukaryotic cells, but a regulation malfunction can lead to various diseases; in fact, over 160 PKs are associated with human diseases such as endocrine disorders, immunodeficiencies and cancers. Numerous experimental approaches for determining specificity between the PK catalytic subunits and their substrates have been done until now, one of them conclude with the identification of a synthetic peptide called Kemptide (Leu-Arg-Arg-Ala-Ser-Leu-Gly) corresponding closely to the phosphohexapeptide sequence reported for pig liver pyruvate kinase, which has good agreement with the kinetic constants of the original substrate. The effect of substituting both Kemptide arginine residues for homoarginine (a non natural aminoacid) have shown an increase of the apparent Km in more than 10-folds in mutated substrates. Therefore, it suggests a great importance of both Kemptide arginines to establish interactions with residues of PK binding site. In this work, we performed free energy perturbation (FEP) calculations(method based on statistical mechanics that is used for computing free energy differences) using previously elaborated MD force field hybrid topology files (under the CHARMM force field) for the unnatural AA homoarginine. Under this method, mutations of both arginines for homoarginines are used to describe the binding affinities of the mutated substrates for PK.

75) Halophilic adaptation of proteins in the archaeal *Methanosarcinales* group

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Organisms that thrive in hypersaline environment are denominated halophiles and are widespread among three kingdoms of life. The halophilic archaea constitutes the classical group of study of halophilic molecular adaptations, particularly the *Halobacteria* group. For these organisms, the accumulation of inorganic ions at molar concentrations, the acidic surface of its proteins associated to a negative electrostatic potential which allows a higher water binding capacity, has been described as the most conserved mechanisms for halophilic adaptation. However, the molecular adaptations to halophilic environments have not been evaluated in the second most important group of halophilic archaea; the order *Methanosarcinales*. In this group, *Methanohalobium evestigatum* is the organism that growth at the highest salt concentrations (up to 5.3 M NaCl). Here we evaluate the halophilic nature and molecular structural adaptations of the ADP-dependent phosphofructokinase/glucokinase from *M. evestigatum* (*Me*PFK-GK) by kinetic, bioinformatics (protein homology modelling) and evolutive (ancestral enzyme reconstruction) studies. The *Me*PFK-GK is stable and active over a wide range of salt concentrations from 0 to 4 M NaCl. Reconstruction of the aminoacidic sequence of the last common ancestor of the *Methanosarcinales* group by Bayesian inference along with computational analysis of the surface of the ancestor and the *Me*PFK-GK showed that both proteins lacks the acidic surface and negative electrostatic potential features observed in proteins of classical halophilic archaea. Our results demonstrate the halophilic nature of the *Me*PFK-GK enzyme and suggest the existence of a new evolutive pathway for the adaptation to hypersaline environments in halophilic archaea.

76) IDENTIFICATION OF AUTOANTIBODIES AGAINST FRUCTOSE-1,6-BISPHOSPHATASE ISOLATED FROM SERUM OF AUTISTIC CHILDREN

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Autism is a complex disease characterized by behavioral deficits, systemic metabolic abnormalities and the presence of serum auto-antibodies targeting key proteins in the brain. Aberrations of brain energy metabolism in autistic patients may involve mitochondrial metabolic dysfunctions within the CNS, a hypothesis supported by frequent manifestations of lactic acidosis in autistic children, apparently caused by a decrease in the rate of lactate utilization in gluconeogenesis. Because the levels of fructose 1,6-bisphosphatase (FBPase) and its substrate are at the core of the gluconeogenic pathway and both might be involved in regulation of cell survival, we hypothesized their link with neurometabolic conditions and symptoms often observed in autistic children. Using immuno-analysis techniques, we found anti-FBPase IgG in autistic patient sera that cross-reacts with liver and muscle FBPase (FBP1 and FBP2). This autoantibody does not affect FBPase enzymatic activity or its susceptibility to AMP, suggesting that the antigenic region is not in the FBP1 functional domains. We also evaluated the cytotoxic effect of these autoantibodies in cell culture and *in vitro* assays using N2a cell line. The results obtained by MTT assay and immuno-active caspase 3 suggest that the presence of anti-FBPase IgG is not harmful to the cell. We conclude that autistic patients have a high level of auto-antibodies, targeting both FBPase isoforms with undetermined metabolic and cellular effects. Nevertheless, the possibility remains that these antibodies may have a differential effect on the FBPase isoforms that disrupts their protein-protein interaction with other structural or metabolic targets.

77) The Plant Growth-Promoting rhizobacteria *Burkholderia phytofirmans* PsJN regulates Arabidopsis growth through a mechanism dependent of gibberellin-related pathways

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Plant growth-promoting rhizobacteria (PGPR) can promote plant growth, reduce susceptibility to diseases and improve tolerance to abiotic stress. Despite the importance of these biological interactions the underlying molecular and physiological mechanisms of growth promotion induced by PGPR are not completely understood. We have previously reported that the PGPR *Burkholderia phytofirmans* PsJN promotes *Arabidopsis thaliana* growth and induces transcriptional changes of genes related with plant hormones biosynthetic and signaling pathways. The objective of this study was to analyze the role of the phytohormones gibberellins (GAs) in the PsJN plant-growth promotion. To tackle this objective we analyzed the spatiotemporal transcriptional patterns of several genes related with GAs biosynthesis and inactivation. Also, the effects of PsJN strain were studied during the whole life cycle of plants mutated in the gene *Ga3Ox1,* which catalyzes the final step in the synthesis of a bioactive gibberellin. We found differential gene expression patterns in shoots and roots of inoculated plants. Some of these genes presented up or down-regulation as soon as 24 hours after inoculation, while other genes were regulated later during the treatment. Also, growth promotion was not detected in the primary roots or rosette areas of the *ga3ox-1* mutants. Taken together, these results suggest that early modulation on GAs signaling/biosynthetic pathway genes and the presence of bioactive GAs are involved in the growth promotion regulated this PGPR strain.

78) Role of microRNAs during maturation of hippocampal neurons

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MicroRNAs (miRNAs) have a fundamental role in the control of both brain development and synaptic plasticity. We have previously described a differential expression of the Polycomb (PRC2)-catalytic subunits Ezh1 and Ezh2, during maturation of rat hippocampal neurons; Ezh2 is found predominantly expressed in immature neurons, whereas Ezh1 is found expressed throughout the maturation process. We have also demonstrated that expression of the PSD-95 gene, which is required to control synaptic plasticity, is increased following Ezh2 knockdown in immature hippocampal cultures, whereas knockdown of Ezh1, decreases PSD-95 transcription in mature hippocampal cultures. To address the contribution of miRNAs during hippocampal maturation and to elucidate the role of miRNAs during regulation of Ezh1, Ezh2 and PSD-95 expression, we have analyzed the global transcriptome and miRNA expression profile of immature and mature rat hippocampal neurons. We generated global miRNA-mRNA interaction network models and subsequently, identified potential regulatory nodes. Additionally, this strategy allowed us to identify a pattern of differentially expressed miRNAs during hippocampal neuron maturation. Particularly important were miRNAs with the ability to target the 3'-UTRs of mRNAs coding for Ezh1, Ezh2, and PSD-95. We show evidence indicating that the differential expression of Ezh2 and Ezh1 and the expression of relevant hippocampal plasticity-related genes that are targets of PRC2, maybe regulated by specific miRNAs during maturation of hippocampal neurons. Our results support a model where miRNA-mediated post-transcriptional mechanisms contribute to regulate the expression of both epigenetic controllers and their downstream target hippocampal genes during the neuronal maturation process.

79) Towards a new representation of DNA chemical diversity to better understand its specificity of recognition by proteins

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DNA molecules are normally represented as letter sequences using a four-letter alphabet. However, a DNA molecule is a double-stranded DNA, where atoms with different chemical properties are exposed at the surfaces of the major grooves. These atoms are strong or weak hydrogen bond donors and acceptors, or a methyl group. The base readout of DNA by proteins involves the specific recognition of the solvent exposed surface atoms lying in the major groove, which nature and arrangement depends on the DNA sequence. In this work, we applied a systematic *in silico* analysis of all possible variants of structures of double-stranded DNA with a length of 4 base pairs. Using the experimental structure of canonical B-DNA as template, we have built full atom 3D models of the 256 duplex DNA variants. Then, different representations of color and physicochemical properties where adopted to visualize the major groove surface, which were either kept in a 3D grid or projected as 2D images. Finally, different metrics were used to calculate the similarities between all possible pairs of DNA duplex molecules, followed by clustering analysis and the generation of the most stable partitions. Our results show that very different molecules at the sequence level can exhibit quite similar physicochemical properties in the major groove surface. This new representation of DNA duplex molecules is important to re-analyze the specificity of protein-DNA recognition from a different perspective, which may prove to be more accurate that the representation of sequences.

80) The orientation of boxes A and B is critical for high affinity binding by MarA and Rob proteins of *mar* and *micF* duplex DNA.

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MarA and Rob are two transcription activators of the *E. Coli* AraC/XylS family, which activate the regulon *marRAB/soxS/rob*. Regulon activation gives resistance to a wide range of antibiotics, superoxide and organic solvents. These two proteins have a DNA binding domain (DBD) with a bipartite Helix-turn-helix (HTH) motif, and Rob also has a regulatory domain (RD). In the case of MarA, H3 and H6 helices interact with the major groove of two regions of DNA called box A (6 bp) and box B (7 bp), respectively. In the case of Rob, only H3 helix from the DBD was found to interact directly with box A. This observation led to the hypothesis that only box A would be required for DNA binding by bipartite HTH proteins. To determine if the presence of box A is the only requirement for binding by Rob-DBD and MarA, Electrophoretic mobility shift assays (EMSAs) analysis were carried out. Our results showed that Rob-DBD and MarA proteins bind to both duplex DNA *mar* and *micF*, while no binding of these proteins to DNA was observed when the boxes A and B were swapped in these two marboxes. This would indicate that the orientation of boxes A and B in *mar* and in *micF* DNA plays an important role in the binding by MarA and Rob-DBD proteins. Further work is required in order to determine if H6 is critical or not in marbox binding or if both boxes are required.

81) The *Piscirickettsia salmonis* LF-89 genome contain two variants of the *hfq* sRNA-chaperone gene

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Piscirickettsia salmonis is the etiological agent of Piscirickettsiosis, a systemic infectious disease that threats the sustainability of salmon production in Chile. *P. salmonis* is a Gram-negative bacterium, and facultative intracellular. To date, the mechanisms of gene regulation has not been studied in *P. salmonis*, but the recently genome sequencing of several strains can help to solve that. In this work was determined the presence of the *hfq* gene, which encodes the Hfq sRNA-Chaperone. Hfq is a protein that binds to sRNA and plays a pivotal role in the regulation of general gene expression in bacteria.

The results from the annotation of *P. salmonis* LF-89 genome, using the RAST server, reveal two ORFs that encodes the Hfq sRNA-chaperone. The alignment of both nucleotide sequence shows that they are not identical, concluding that this organism contain two *hfq* variants. Protein alignment confirms that both sequence conserve the typical domains implicated in sRNA binding. The structural model of both proteins, obtained with the I-TASSER server, shows a highly structural homology between them, which is conserved with other Hfq proteins. Finally, interaction analyses by protein-protein docking, reveals that both Hfqs are capable to form an hexameric-ring structure, which is the functional form.

In summary, our results demonstrate that *P. salmonis* encodes two highly conserved *hfq* genes. Only in *Burkholderia cepacia* has been described two Hfq variants, suggesting that a complex regulation in the expression of both *hfq* genes might exist in order to modulate fitness and virulence of the bacteria.

82) Polycystin 2 is required for rapamycin-induced atrophy

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Background: Muscle volume adapts to different pathophysiological conditions activating pathways that regulate protein degradation. AIDS, cancer, sepsis, heart failure, burn injury and cancer pharmacological treatments can induce severe muscle atrophy. Autophagy, the major catabolic intracellular mechanism, is involved in muscle wasting during atrophy. Polycystin 2 (PC2) has been previously identified as a protein able to regulate tissue cell size through regulation of AMPK and mTOR, which are major regulators of autophagy. We hypothesized that Polycystin 2 is required for skeletal muscle atrophy in myotubes C2C12 and L6. ***Methods and Results***: Treatment with rapamycin 10 mM for 4 h induced atrophy and autophagy in myotubes C2C12 and L6, as suggested by the significant decrease in the level of myogenic markers (myogenin and caveolin-3), and increased lipidation of LC3, respectively. PC2 downregulation, using specific siRNA, prevented both rapamycin-induced atrophy and autophagy. Consistently, PC2 over-expression induced atrophy and autophagy in C2C12 and L6 myotubes. ***Conclusion:*** These results suggest that PC2 regulates atrophy and autophagy in C2C12 and L6 skeletal muscle cells.

83) Polycystin-2 regulates autophagy through the mTOR/AMPK pathway in skeletal muscle cells

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Background: Autophagy is a lysosomal degradation pathway for proteins and organelles required to maintain cellular homeostasis. mTOR and AMPK are nutrient sensor proteins that inhibits and activates autophagy, respectively. Polycystin-2 (PC2) is a transient receptor potential channel and mutations in this gene (*pkd2*) cause polycystic kidney disease (PKD). Studies have shown increased mTOR activity in kidney of PKD patients and treatment with rapamycin improves PKD symptoms and reduces cyst formation in an animal model of PKD. We hypothesized that PC2 regulates autophagy through mTOR/AMPK pathway in C2C12 and L6. ***Methods and Results:*** Autophagy was induced in C2C12 and L6 with 1 mM rapamycin or nutrient deprivation (starvation). Using specific siRNA against PC2 we found that PC2 is necessary for rapamycin- or starvation-induced autophagy in C2C12 and L6, as assessed by LC3I to LC3II conversion and GFP-LC3 dots formation. Furthermore, mTOR and AMPK were evaluated in cells knock-down for PC2 following autophagy induction. Our results show that PC2 knock-down prevents rapamaycin- and starvation-induced mTOR inhibition, as shown by western blot by increased phosphorylation levels of mTOR, S6 and 4EBP1. Consistently, PC2 knock-down inhibits starvation-induced AMPK activation. ***Conclusion:***Our results suggest that PC2 regulates autophagy in myotubes L6 and C2C12 through the mTOR/AMPK pathway. This work was supported by FONDECYT grant 1140908.

84) Unveiling novel extra circadian functions for the oscillatory clock protein

Frequency in *Botrytis cinerea*.

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Botrytis cinerea is a necrotrophic fungus that infects over 200 plant species and is considered the second most important phytopathogen due to its scientific and agronomic importance. We have demonstrated, for the first time, the presence of a functional circadian clock in a pathogenic organism. Genetic disruption of the *B. cinerea* oscillator by deletion of the core negative element (BcFRQ1), by its overexpression or by suppression of the rhythmicity by constant light, abrogates circadian regulation of fungal virulence. Remarkably, the *bcfrq1* knockout strain has a strong impact on sexual/asexual reproduction, which is further modulated by modifying media culture conditions. This differs with what has been reported for the *frq* knockout strain of *Neurospora crassa*- the only other fungus in which a circadian clock has been dissected- which shows no reported phenotype related to conidia production. Importantly, the developmental phenotype of the*bcfrq1* mutant is observed even under conditions where the clock is already disrupted by a constant environmental cue, suggesting extra-circadian functions for BcFRQ1. In order to explore these new-BcFRQ1 roles, we have performed global gene expression analyses of the mutant. Functional categorization of the genes depicting changes allowed us to identify several metabolic-related genes overrepresented among those being down-regulated. Remarkably, we identified an important deficiency in secondary nitrogen metabolism and/or uptake that correlates with the mentioned developmental phenotype. We are now examining defects in signaling pathways and developmental-specific genes in order to elucidate the mechanisms behind the mentioned phenomena. FONDECYT 1131030 and Millennium Nucleus NC120043.

85) ENDOTHELIN-CONVERTING ENZYME-1C PROMOTES COLON CANCER AGGRESSIVENESS: NEW INSIGTHS FOR AN ET-1 INDEPENDENT EFFECT

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Introduction. Endothelin-Converting Enzyme-1c (ECE-1c) is a membrane Zn-metalloprotease that synthesizes Endothelin-1 (ET-1). In many cancers, ET-1 has mitogenic properties, promoting proliferation and invasion. In addition, ECE-1c has been observed as a mediator of cancer invasiveness, however, whether this process is ET-1-dependent is unknown. Thus, we studied the role of ECE-1c and its ET-1-dependency in the development of malignant characteristics in colon cancer cells. **Material and Methods.** ECE-1c was overexpressed in DLD-1 cells by lentiviral infection, and genetic silencing was performed by sh-RNA. Cell viability, migration, invasion and anchorage-independent proliferation were evaluated by MTS, transwell, matrigel and soft agar, respectively. FAK and Akt activation were evaluated by western blot. TPA was used to activate PKC and 5-Fluorouracil as death inducer. Subcelular localization experiments was performed by confocal microscopy and imaging analysis using ImageJ. Tumor growth was evaluated by subcutaneous tumor formation in NOD/SCID mice. **Results.** ECE-1c promoted activation of FAK and Akt, correlating with an increased migration, invasion and anchorage-independent proliferation. Cells overexpressing ECE-1c showed resistance to 5-Fluorouracil compared to control. ECE-1c overexpression in ET-1 silenced cells showed similar increase in migration, as well as conferred resistance to ET_AR and PI3K inhibitors compared to control. Tumor growth *in vivo* was significantly augmented by ECE-1c overexpression. TPA treatment led to NT-ECE-1c-GFP to localize to plasma membrane. **Conclusions.** ECE-1c promotes malignant characteristics in colon cancer cells *in vitro* and *in vivo.* Our findings suggest an ET-1-independent mechanism by which ECE-1c promotes colon cancer cell survival and migration. **Acknowledgments. FONDECYT.grant 1120132,** CONICYT.PhD.fellowship 21130753.

86) Insights of cold adaptation mechanisms in proteins assessing the flexibility of the psycrophilic and mesophilic ADP-PFK/GK from *M. burtonii* and *M. maripaludis*

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Psychrophilic enzymes are able to perform its function at low temperatures which has been attributed to an improvement of the dynamics or the flexibility of their structures. Members of the ADP-dependent sugar kinase family inhabit a diverse range of environmental temperatures and represent a good model for studying structural determinants of cold adaptation in proteins. The structure of these enzymes is composed of two domains (major and minor) which are connected by a flexible region (hinge). Domain movement associated to substrate binding and catalysis has been described for several members of this family. In this study, we aimed to gain insights regarding the mechanism of cold adaptation used by the psycrophilic ADP-dependent phosphofructokinase/glucokinase (PFK/GK) from *Methanococcoides burtonii*. For this purpose we engineer the psycrophilic ADP-dependent PFK/GK from *M. burtonii* and the mesophilic homolog enzyme from *Methanococcus maripaludis* in order to introduce tryptophan (Trp) residues in specific regions of the protein to assess protein flexibility by acrylamide fluorescence quenching. One of the Trp residues was located in the hinge and the other in the major domain. For the *M. maripaludis* enzyme these positions corresponds to N203W and V231W while for the *M. burtonii* enzyme these positions corresponds to W214 and Y242W, respectively. Kinetic characterization of both ADP-dependent PFK/GK mutants indicates that the Km and Vmax values for ADP and fructose-6-P are very similar to those of the wild type enzymes. Fluorescence experiments show that both mutants sense ligand binding and can be used to probe the accessibility of tryptophan residues.

87) Intrisical disorder and amyloid aggregation inhibition of the antimicrobial protein Microcin E492.

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Amyloid aggregation is a widespread phenomenon that also been observed in microorganisms. Bacterial amyloids have been associated with several functions such as adhesion factors or host response inhibitors. Microcin E492 (MceA) is an antimicrobial protein of 84 residues produced by *K. pnumoniae* RYC492, resistant to some proteases, extreme pH and heat. Its mechanism of action involves a specific recognition of the target by a postraslational modification (PTM) molecule that is attached at C-terminal of MceA. MceA has the ability to forms amyloids that sequester toxic species, process that is retarded by PTM. The structural nature of the inhibition of aggregation is not well understood. In this work, characterized MceA using size exclusion chromatography (SEC), circular dichroism (CD) and differential scanning fluorimetry and calorimetry (DSF, DSC). Our results show that isolated soluble MceA is principally a monor in a partially disordered state. *De Novo* prediction shows that monomer contains secondary structure motifs and hydrodynamic radius in agreement with our experimental results. CD spectra after titration of MceA with Trifluoroethanol show that helical and beta contents are competing features modulated by PTM. By sequence analysis we detect five proamyloidogenic regions in MceA. Deletion of the 54-63 region showed a remarkable decrease of amyloid formation followed by Thioflavin T and AFM. These results suggest that mature MceA is an intrinsically disordered protein where PTM affects the equilibrium between helical and beta structures, wich seems to be a mechanism to control amyloid aggregation. Suported by FONDECYT 1140430

88) Cell metabolism increases in the early stage of mitochondrial unfolded protein response in HeLa cells

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Introduction: Cell homeostasis is maintained by responding to changes in different variables through feedback responses that involve modifications in gene expression and metabolism to support cell survival. Protein homeostasis is regulated by genetic programs named unfolded protein responses (UPRs) that maintain the balance between the load of client proteins and protein handling capacity. Mitochondria are susceptible to accumulation of misfolded/damaged proteins due to their pro-oxidant environment, the requirement of coordinated nuclear/mitochondrial gene expression and the import of proteins from the cytosol. Mitochondrial UPR (UPR^{mt}) has an elevated energetic requirement, but it remains unknown whether this response involves metabolic changes. **Aim:** The aim of this work was to evaluate if the response to mitochondrial proteotoxic stress involves a metabolic component to support homeostasis. **Methodology:** We use as model of UPR^{mt}, HeLa cells treated with doxycycline (15 µg/mL) to alter mitochondrial translation and induce proteotoxic stress. UPR^{mt} markers were analyzed by qPCR. Mitochondrial membrane potential ($\Delta\psi mt$) was measured by TMRM-staining and flow cytometry. Intracellular ATP levels were measured with a luminescence-based commercial kit. **Results:** HeLa cells treated with doxycycline showed an increased expression of UPR^{mt} markers with a peak between 4-8 h of treatment. They also exhibited increased ATP levels with a maximum at 2 h. However, we were not able to detect changes in $\Delta\psi mt$. **Conclusions:** Intracellular ATP levels increase during the early stage of proteotoxic mitochondrial stress in HeLa cells. However, it remains unexplored if this change depends on a boost in mitochondrial metabolism.

89) Glutathione depletion induces UPR and autophagy in germ cells

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Glutathione (GSH) plays an important role in the antioxidant defenses of the spermatogenic epithelium. Autophagy is the major intracellular degradation system and acts as a pro-survival response during several conditions. This process is upregulated in response to ER stress and antioxidant imbalance. In this work, we evaluated whether autophagy through UPR (Unfolding Proteins Response) is involved in spermatogonia-type germ cell (GC-1) survival during GSH depletion. We showed that disruption of GSH metabolism with L-buthionine-(S,R)-sulfoximine (BSO) decreased GSH content in GC-1 cells, without altering ROS production and cell viability. An increase of BIP and CHOP protein levels was observed in GSH-depleted cells. Autophagy was assessed by processing the protein LC3I to LC3II and observing its sub-cellular distribution. Immunoblot and immunofluorescence analyses showed a consistent increase in LC3II levels and accumulation of autophagosome under GSH-depletion conditions. This process did not affect the activity of AMP-activated protein kinase (AMPK) or the ATP content. However, inhibition of autophagy resulted in decreased ATP content and increased caspase-3/7 activity in GSH-depleted GC-1 cells. Finally we evaluated GSH level, UPR and autophagy induction in a pool of germ cells in primary culture. These findings suggest that GSH deficiency triggers an AMPK-independent autophagy through an UPR induction.

90) A Method to Determinate Functional HREs on Genes Regulated by Hif-1.

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Hypoxia is a reduction in the normal oxygen tension. A key regulator factor involved in hypoxia cell response is the hypoxia-inducible transcription factor-1 (HIF-1). The stabilization is involved in Epithelial-to-Mesenchymal Transition (EMT) by direct bind to hypoxia response elements (HREs) on its target genes, being one of them SNAIL, an E-cadherin (E-cad) repressor. A second sequence near to HRE, HIF Ancillary Sequence (HAS), has been showed as important in the activity transcriptional of HIF-1. We recently published that Hif-1 controls neural crest cells (NCCs) EMT by down-regulating E-cadherin in *Xenopus laevis*. Previous data indicate that also zebrafish E-cadherin needs to be down regulated to allow NCCs migration. Despite this, transcriptional repressors regulated by Hif-1 in zebrafish NCCs EMT, remain unknown. Using bioinformatics toolswe want to identify functional HREs in genes that regulate NCC EMT. To demonstrate that *snail1b* was regulated by Hif-1α in zebrafish, we did assays of loss- and gain-of-function and analyze by qPCR and *in situ* hibridization the expression pattern of *snail1b*. Then we used Chromatin Immune Precipitation (ChIP) to determine the presence of Hif-1α in the regulatory region on *snail1b* gene. Our results evidence a direct regulation of *snail1b* by Hif-1α during NCCs development. Funding FONDECYT 1150816, CONICYT/FONDAP 15110027.

91) Developing Nanoparticles Towards the Ultrasensitive Detection of Cancer Biomarkers

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Gastric cancer remains the second deadliest type of cancer worldwide and its rapid progress and lack of specific symptoms make it necessary to find new early detection and prevention methods. We aim to use bifunctional gold nanoparticles towards this end, firstly by attaching an oligonucleotide to the nanoparticle, which gives it specificity (by base complementarity) to a certain sequence of the Reprimo gene, which has been described as an early gastric cancer biomarker when methylated. Second, we add a Raman reporter molecule which makes the nanoparticle visible to a Raman microscope, allowing for the visualization of the molecule through surface-enhanced Raman spectroscopy (SERS), which is an analytical technique with very high sensitivity, capable of reaching single-molecule detection under certain circumstances. The system is complemented by the use of magnetic iron oxide nanoparticles, functionalized with another oligonucleotide which is also complementary to another part of the Reprimo gene sequence. The system of hybridized gold nanoparticle-iron oxide nanoparticle-methylated DNA can later be captured by using a magnet and processed for quantification through SERS and other techniques like Real Time PCR or BEAMing. Here we report on the progress of the development of these nanosystems for detection of Cancer Biomarkers.

92) Biomimetics: From Bioinformatics to Rational Design of Dendrimers as Gene Carriers

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Biomimetics, or the use of principles of Nature for developing new materials, could help Nanomedicine to solve new paradigms. One of the current challenges in Nanomedicine is the rational design of new efficient and safer gene carriers. Poly(amidoamine) (PAMAM) dendrimers are a well-known class of nanoparticle, extensively used as non-viral nucleic acid carriers, due to their positively charged end-groups. In spite of that, several factors have to be improved for their successful application in in vitro and in vivo systems, associated to the affinity for nucleic acids and at the same time, avoid cytotoxicity induction. In the search of new functional groups that could be used as new dendrimer-reactive groups, we followed a biomimetic approach, to determine the amino acids with higher prevalence in the Protein-DNA contact zone, and later, introduce them or a mixture of them as terminal groups of dendrimers, generating a new class of nanoparticle. Moreover, molecular dynamics studies of two systems: PAMAM-Arg and PAMAM-Lys were also performed in order to describe the formation of complexes with DNA. Results confirmed that the introduction of amino acids as terminal groups of a dendrimer might result in promising new gene carriers.

93) IDENTIFICATION AND TRANSCRIPTIONAL ANALYSIS OF EXPANSINS IN THE MOLECULAR RESPONSE TO INCLINATION OF RADIATA PINE.

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Plants have the ability to reorient their vertical growth when are subjected to inclination. In nature, conifer trees develop compression wood in response to gravitropic stimuli in the lower side of the stem. The genes involved and the molecular mechanisms under this phenomenon are still unknown. Several studies have identified genes encoding proteins named expansins, which are involved in cell expansion process among other developmental biological processes in vegetal organisms. Therefore, in order to provide new data relating to these proteins in this biological phenomenon of inclination response in radiata pine seedlings, libraries obtained by sequencing of RNA (RNAseq) at different times of inclination were analyzed. From those libraries, 22 putative sequences was identified and classified as expansins. In order to validate these sequences and have a first approach of their participation in this phenomenon, a transcriptomic profiling of 6 genes coding for expansins were analyzed by qPCR. Results showed an increased expression in the lower side of stem and a repression of one of those genes under the gravitropic stimulus. In addition, the sequence showing the highest levels of accumulation and a high level of identity to alpha expansins, was three-dimensionally modeled in order to determine whether it exhibits the characteristic structural motifs of these proteins. Results showed that PrEXPA4 has a tertiary structure consisting in two highly conserved domains with an open groove between them, six cysteines which form disulfide bonds and characteristic motif composed of His-Phe-Asp amino acids (HFD). All these results suggest that expansins proteins could be taking part in the gravitropic response and may have an important role in the formation of compression wood in radiata pine.

94) Calcium sensing receptor activation increases FABP4/aP2 protein and autophagy gene expression in human LS14 adipocytes and adipose tissue

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We have previously shown that stimulation of the G-protein coupled extracellular calcium sensing receptor (CaSR) is associated with elevated proinflammatory factor expression (IL1-beta, TNF-alpha, IL6, CCL2) in LS14 human adipocytes and adipose tissue (AT), proposing a role for the CaSR in inflammation-induced AT dysfunction. It has been recently described that AT dysfunction and inflammation in obesity are associated with elevated levels of the adipocyte differentiation marker fatty acid binding protein 4 (FABP4) in AT and plasma. Obesity and AT dysfunction have been also linked to elevated AT autophagy, however the association between FABP4 and autophagy is still unclear. This work evaluated the role of CaSR activation on FABP4 expression and protein content in human LS14 adipocytes and human AT. We observed an increase in FABP4 gene expression in LS14 adipocytes exposed to the CaSR allosteric activator cinacalcet (2mM) for 24 hours and a dose-dependent (1, 2, 5 mM) increase in FABP4 protein in AT from obese subjects treated ex-vivo. In addition, we evaluated the expression of autophagy genes to assess their possible role mediating the CaSR effect on FABP4. Exposure of LS14 adipocytes to cinacalcet (2mM) elevated mRNA expression of the autophagy markers Atg5, LC3A y LC3B. Autophagy inhibition by chloroquine (50mM) or bafilomycin (10nM) prevented the cinacalcet-induced elevation in FABP4 mRNA expression. Together, our data suggest that CaSR activation elevates FABP4 in human adipose cells, and this effect could be mediated by elevated autophagy. PM holds a PhD CONICYT fellowship. FONDECYT 1150651 (MC), FONDAP 15130011 (SL)

95) Domain swapping of the DNA-binding domain of human FoxP1 is facilitated by its low folding stability.

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Protein folding and oligomerization are biologically relevant processes when reaching the quaternary structure is required for function. Proteins that form dimers by exchanging segments of their tertiary structure with another subunit (domain swapping) are examples where folding and dimerization are concerted processes. Previous studies of domain swapping have shown that, in general, a high kinetic barrier separates monomers and domain swapped dimers, but protein unfolding and refolding at high protein concentrations favor the swapped oligomer. Recently solved structures of the DNA-binding domain of the P subfamily of human forkhead box (FoxP) proteins show the presence of swapped dimers. These transcription factors are interesting models of domain swapping, since mutations in the hinge region that connects the exchanged segment with the rest of the protein are linked to inherited disorders, such as IPEX and language deficits. Also, FoxP1 and FoxP2 can reach monomer-dimer equilibrium in solution after hours of incubation, suggesting that a low kinetic barrier separates both species. Using FoxP1 as a model of domain swapping, we analyzed the temperature and protein concentration effects on the dimer dissociation, obtaining the free energy change and enthalpy of the process by vant Hoff analysis (ΔH^0 = 23.1 kcal/mol, ΔS^0 = 0.082 kcal/mol/K and ΔG^0 = -0.95 kcal/mol at 25º C). These results indicate that dimer formation is an example of an enthalpy-driven process. To understand how FoxP1 domains swap without protein unfolding, we performed equilibrium unfolding experiments using GndHCl as denaturant, showing that the wild-type (wt) protein has a low stability (ΔGU = 6 kcal/mol, Cm = 3.5 M at 25º C), in contrast to other domain swapping proteins with high kinetic barriers. We explored the domain swapping mechanism of FoxP1 through molecular dynamics simulations, showing that the exchange process can occur by local destabilization and unfolding of the hinge region and helix H3. To investigate if the low stability of wt FoxP1 facilitates its domain swapping, we engineered a single-point monomeric mutant of FoxP1, and used this protein to visualize the effect of monomer stability in dimer formation. Comparison of the folding stability of the A39P mutant and wt FoxP1 shows that ΔΔGU (mut-wt) is ~2.5 kcal/mol, concluding that the ability of FoxP1 to domain swap rapidly can be explained by its low monomer stability and local unfolding.

96) *De novo* assembly characterization and analysis of fine flounder (*Paralichthys adspersus*) skeletal muscle transcriptome under conditions of nutritional stress

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Introduction: The *Paralichthys adspersus* is an endemic species of greatest farming potential. However, its farming potential is limited because of their slow growth rates; therefore the viable cultivation of this species requires new strategies to improve growth rates. Next generation sequencing (NGS) technologies has revolutionized the field of transcriptomics by the analysis of the global differential gene expression. Thus, the aim of this work use RNA-seq for analyzes the reprogramming of muscle gene expression triggered by contrasting nutritional status.

Methods:Three paired-end libraries were generated from RNA samples obtained from skeletal muscle of fine flounder, which were subjected to 3 weeks of starvation then to 1 week of refeeding. Control was defined as sample prior to the start of the starvation. Each library was sequenced twice by MiSeq of Illumina. Next, the reads obtained were analyzed and filtered by quality. Trinity software was used for *de novo* transcriptome assembly. Finally, the mapping and transcript abundance estimate per condition has been made.

Results: We obtained a total of 44 million reads. These reads were assembled into 74,626 transcripts with an average length of 1,235bp, N50 value of 2,061 and the coverage of transcriptome was 48X. We found that around 8,000 transcripts were expressed exclusively in starvation and around 10,000 were expressed exclusively in refeeding.

Discussion: We generated a comprehensive first reference transcriptome of fine flounder skeletal muscle. Our preliminary results show several genes change their expression between conditions. Our work provides a transcriptomic resource for future gene expression analysis in this species.

97) Angiotensin-(1-9) decreases cardiomyocyte death triggered by ischemia/ reperfusion

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Introduction: Ischemic heart disease is the most common type of cardiovascular pathology and its impact is due to cardiomyocyte death. Cardiomyocytes are responsible for heart contractility, they are unable to proliferate and their loss lead to heart failure. Myocardial ischemia and reperfusion(I/R) generate cardiomyocyte death by apoptosis and necrosis. In recent years, a “non-canonical” renin angiotensin system has been discovered in which Angiotensin-(1-7) (Ang-(1-7) via MasR and Angiotensin-(1-9) (Ang-(1-9) via AT2R antagonize the deleterious action of Ang II. Ang(19) has shown anti-hypertrophic effects in heart, however, it is unknown whether Ang-(1-9) has cardioprotective actions during I/R. **Objective:**to assess whether Ang-(1-9) decreases cardiomyocyte death triggered by I/R. **Methodology:** Cultured rat cardiomyocytes were stimulated to I/R in the presence or absence of Ang-(1-9) as well as the MasR antagonist (A779). Parallel controls were incubated under normal conditions, were reperfused and subjected to the same treatments. Cell death was evaluated by lactate dehydrogenase (LDH) release, population in Sub-G1 phase and cleaved caspase-3 by immunofluorescence. **Results:** Cardiomyocytes subjected to I/R compared to control cells showed an increase of LDH release (from 18% to 45%), percentage of cells in sub-G1 (from 4 to 17%) and expression of cleaved caspase-3 (from 10 to 60%). Ang(19) significantly decreased all these parameters ($n=3$, $p<0.05$) and they were not modified by the co-incubation of Ang-(1-9) plus A779. **Conclusion:** These results suggest that Ang-(1-9) decreases cardiomyocyte death during I/R and these effects are not mediated by Ang-(1-7)/MasR. **Acknowledgment:**EM hold a PhD CONICYT fellowship. FONDEF D11I1122(MPO;SL), FONDAP15130011(MPO;SL).

98) Angiotensin II induces autophagy in vascular smooth muscle cells

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Vascular smooth muscle cells (VSMCs) are an essential component of vessels involved in vascular tone regulation. During hypertension, VSMCs change from a contractile to a proliferative phenotype and facilitates the development of vascular remodeling and cardiovascular diseases. Here we evaluate the effect of Angiotensin II (Ang II) on autophagy as responsible for phenotype switching in VSMCs. Smooth muscle cells A7r5 from rat aorta were treated with Ang II (100 nM) for 24 h. Autophagy was evaluated by LC3II/GAPDH, autophagic flux and confocal microscopy with adenovirus LC3-GFP in the presence and absence of chloroquine. Ang II induces autophagy at 24 h by increasing the LC3II/GAPDH ratio, autophagic flux and the number of LC3 II positive vesicles (autophagic dott). Our data suggest that Ang II induces autophagy in VSMCs. This process could play a key role in VSMC phenotype switching induced by Ang II in cardiovascular diseases.

99) Combining mass spectrometry, *in silico* collision-induced dissociation and molecular dynamics simulations to analyse intact membrane proteins under vacuum.

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Structural biology on membrane proteins has been an evolving field over the years. Purified membrane proteins in solution are usually complexed to detergent micelles to maintain their solubility. Recently we have discovered a new method based on Ion Mobility Mass Spectrometry (IM-MS) to analyse the structure of intact membrane proteins in the gas phase. This process involves first the transfer of membrane protein-micelle complexes (MPMCs) from solution into gas phase and second the release of the protein after MPMCs are collided with gas molecules at high kinetic velocity to induce selective removal of the micelle. However the precise molecular mechanism of this process is unknown. We present three computational methods to analyse this process on drug transporters and ion channels. First, increasing the temperature on molecular dynamics (MD) simulations in gas phase, this process is analogous to the protein internal energy increase occurred within the mass spectrometer. Second, applying a probabilistic model based on MPMC geometrical properties to the transfer of internal energy occurring after collision. Using the solvent accessible surface area (SASA) calculated for each residue and detergent molecule in the complex, we could estimate a higher probability of collision at larger SASA values. Third, analysing the collision events via nano-mechanical simulations of the MPMCs collided to gas molecules. Combining IM-MS with the computational methods, a mechanism of the membrane protein stability is postulated. We anticipate that these results will guide future experiments to make suitable MPMCs for structural studies under vacuum.

100) Integrative inference of transcriptional regulatory networks in a model eukaryote

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Transcription factors (TFs) lie at the core of gene regulatory networks and their concerted and combinatorial interactions with cis-regulatory modules contribute to the precise temporal and spatial regulation of gene expression. The filamentous fungus *Neurospora crassa* has a long-standing history as a model organism for the study of gene regulation, yet little is known about the function of the vast majority of its TFs, their DNA-binding preferences and the regulatory networks in which they participate. To address this issue, we first used protein binding microarrays to determine the DNA binding preferences of *N. crassa* TFs, obtaining motifs for 146 (~54% of *N. crassa* TFs), representing more than a 10 fold increase in known binding specificities in this fungi. This placed *N. crassa* as the filamentous fungi with the highest motif coverage and as one of the organisms with the highest number of experimentally determined motifs among eukaryotes. By applying state-of-the-art network inference algorithms, we then integrated this data and publicly available microarray and RNA-seq datasets to infer transcriptional regulatory networks in *N. crassa.* We are using these networks to predict the function of specific TFs in various processes and generate novel hypotheses of regulatory programs in this fungus, several of which we are currently experimentally evaluating. Our inferred regulatory networks provide a global view of the regulatory circuitry underlying gene expression in *N. crassa* and we anticipate them to be a powerful resource for functional genomics and the study of gene regulation in filamentous fungi.

101) 3D pharmacophore searching and selectivity analysis on a series of anti-tuberculosis compounds associated to the protein kinases B and G: *pharmacophore* based virtual screening.

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Among the main aims of the United Nations is to eradicate communicable diseases such as tuberculosis, which affect millions of people worldwide producing more critical problems in countries with low- and middle-income. For this reason, a study involved *3D pharmacophore* searching, selectivity analysis and virtual screening for a series of anti-tuberculosis compounds associated to the protein kinases B and G was development. This theoretical study is expected to shed some light onto some molecular aspects that could contribute to the knowledge of the molecular mechanics behind interactions of these molecules with anti-tuberculosis activity. Using the molecular quantum similarity (MQS) field and reactivity descriptors supported in the Density Functional Theory was possible the quantification of the steric and electrostatic effects through of the Coulomb and Overlap quantitative convergence scales (alpha and beta). In addition, an analysis of reactivity indexes is development, using global and local descriptors, identifying the binding sites and selectivity on the anti-tuberculosis compounds. Finally, the pharmacophores to PKn B and G reported were used to carry out virtual screening using a database with anti-tuberculosis drugs, to found the compounds that can have affinity by the specific protein targets associated to PKn B and G, respectively.

102) Differential expression of lncRNAs during osteogenesis.

Nardocci, Gino[1,2]., Carrasco, Margarita[2,1]., Torres, Gustavo[2,1]., Acevedo, Elvis[2,1]., Meneses, Claudio[3]., Montecino, Martín[2,1]., [1]Center for Genome Regulation FONDAP. [2]Center for Biomedical Research, Faculty of Biological Sciences and Faculty of Medicine, Universidad Andrés Bello.[3]Center of Plant Biotechnology, Faculty of Biological Sciences, Universidad Andrés Bello. (Sponsored by FONDAP-15090007; FONDECYT-1130706; FONDECYT-3140414.)

Long non-coding RNAs (lncRNA) are a type of non-coding RNAs that are longer than 200 nucleotides, 5`capped, polyadenylated, and poorly conserved among mammalian species. Their expression is developmentally regulated and can be tissue- and cell-type specific. LncRNAs are a relatively abundant component of the mammalian transcriptome and have been implicated in several cellular functions including regulation of chromatin structure, control of mRNA translation, regulation of gene transcription, and regulation of embryonic pluripotency and differentiation. Nevertheless, the precise mechanisms by which lncRNAs control gene expression are not yet understood. Recent advances in the methodologies utilized to analyze in depth the complexities of the transcriptome have unveiled the existence of new lncRNAs with relevant roles 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 isolated 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 sequences were aligned and transcript abundances determined. New lncRNA candidates that displayed a differential expression pattern during the osteoblast differentiation process were identified. We then evaluated the presence of these lncRNAs in different mouse tissues, observing cell-specific abundance. A group of these lncRNAs also showed reduced enrichment in other mesenchymal-derived tissues, including muscle. Importantly, the expression of these lncRNAs was confirmed in both osteoblastic and muscle mouse model cell lines, exhibiting specific expression profiles.

103) Molecular characterization of the interaction of a COld regulate (COR) protein and a series of thylakoid mixture membranes in response of cellular dehydration by Coarse Grained Molecular Dynamics (CGMD) simulations

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Abiotic stresses, such as drought and cold markedly retard growth of plants. To protect cells under these stresses, plants produce proteins like COld Response (COR) proteins, where the best characterized correspond to the chloroplast amphipathic protein COR15A. Several studies have been realized in order to quantify the protective role of COR15A in thylakoid membranes. They showed that the structural order of the protein is a crucial parameter for lipid protection, where they suggest that the protection occurs by the adsorption of COR15A peripherally into the headgroups of the bilayer.

To analyze this process several CGMD simulations were performed, using different conformations of COR15A: Folded, partially folded and unfolded in the presence of mixtures of thylakoid lipids: monogalactosyldiacylglycerol, digalactosyldiacylglycerol and sulfoquinovosyldiacylglycerol. At the same time, using the ordered conformation of COR15A, we studied importance of the unsaturation degree in the adsorption of COR15A in the bilayer.

Based in the simulations, we found that the more ordered protein is able to interact in a better way than the partially folded and the unfolded conformation as the experimental data shows. We suggest that this occur based in its amphipathic nature, where the more ordered posses a greater spectrum to interact by its hydrophilic interface with the headgroup of the thylakoid lipids, than the others conformations. We also found that the degree of unsaturation isn't determinant in the interaction with COR15A, where in the presence of either saturated or unsaturated lipids COR15A stay mainly interacting to the headgroups of the bilayer.

104) ECE-1C UBIQUITINATION AND ITS ROLE IN COLON CANCER INVASION

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Background: CK2 phosphorylates over 300 proteins, regulating essential cellular processes, most of them with key importance in cancer. Endothelin Converting Enzyme-1c (ECE-1c) is a membrane metalloprotease that synthesizes endothelin-1 which may act as mitogen to promote cancer progression and metastasis. We have previously demonstrated that N-terminal of ECE-1c is phosphorylated by CK2 which enhances its stabilization. In this work, we evaluated whether CK2-dependent phosphorylation prevents ECE-1c ubiquitination and subsequent UPS-mediated degradation, as well as its implication in colon cancer cell migration and invasion**. Material and Methods:** Protein stability was measured with the cycloheximide assay in CHO-K1 cells using TBB as a CK2 specific inhibitor. Ubiquitination was evaluated by pull-down assay in CHO-K1 cells overexpressing His-tagged Ubiquitin. Migration and invasion were analyzed in DLD-1 colon cancer cells by both transwell and matrigel assays, respectively. **Results:** Inhibition of CK2 promoted ECE-1c degradation. Also, CK2-dependent phosphorylation of ECE-1c improved the migratory and invasiveness capabilities of colon cancer cells. **Conclusions**: CK2-dependent phosphorylation at the N-terminal end of ECE-1c promotes its stability through blockage of UPS-mediated degradation, which improves migration and invasion of colon cancer cells.

105) Molecular detection and genotypification of *Helicobacter pylori* in stool samples from symptomatic adult patients in Coquimbo, Chile.

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Introduction: Infection for *Helicobacter pylori* is the main risk factor for the development of gastritis and peptic ulcer, moreover of gastric cancer. In Chile 76.8% of the population is infected.

The objective of this study was to develop a diagnostic method based on nested-qPCR to detect the *H. pylori* gene, ureC, and determine the prevalence of cagA+ strains among the infected patients.

Methodology: DNA was isolated from stool samples of 53 digestive symptomatic patients, and then nested qPCR was applied for the amplification of the ureC and cagA genes. RUT (Rapid Urease Test) and histology with Giemsa stain were indicated for each one of patients.

Results: In the clinical practice the sensitivity of RUT to detect *H. pylori* infection was only 57%, while the specificity was 75%, meanwhile the positive predictive value (PPV) was 97% and the negative predictive value (NPV) was 12%. For other hand, the sensitivity of our test based on nested-qPCR was 100% while the specificity was 81%. The PPV NPV were 98 % and 100% respectively.

Between the 48 patients positive for H. pylori, 40 were positive for cagA+ strains (83.3%), which is in concordance with the high prevalence of gastric cancer in Chile.

Conclusions: We found in this work, that nested-qPCR is better than RUT in to detect the presence of infection by *H. pylori*. Moreover, we can to detect the presence of more aggressive strains by a simple and non-invasive test.

106) ADENOSINE MEDIATES INDUCTION OF RENAL AMINOPEPTIDASE A IN EXPERIMENTAL DIABETES MELLITUS

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Introduction. Diabetic nephropathy (DN) remains to be the primary cause of terminal renal disease. Progression of DN is linked to intrarenal induction of the renin-angiotensin system (RAS). The activity of RAS is dependent on peptidases that cleavage the precursor angiotensin to generate peptides with diverse detrimental effects, both at renal and cardiac tissues. DN also progresses with elevated levels of adenosine. Thus, our aim was to study the crosstalk between adenosine signaling and RAS activity.

Metodology. Glomeruli were isolated from rat kidney by a differential sieving method and treated ex vivo with adenosine 10µM or a set of pharmacological modulators of adenosine receptors in HAMF10, at 37°C for 24 h. Differences in protein composition of glomeruli were determined by comparative proteomic. The aminopeptidase A (APA) content was evaluated by western blot in total protein extracts. Adenosine deaminase (ADA) was administered to streptozotocin-induced diabetic rats at 5U/week for 1 month.

Results. Increased APA content was evidenced by using comparative proteomic between glomeruli exposed to adenosine and untreated. The induction of APA was confirmed by western blot analysis in adenosine-treated glomeruli and in kidney from diabetic rats. The increased renal content of APA was reverted by treatment of diabetic rats with ADA, which prevented adenosine receptors signaling. Using pharmacological modulators we determined that the induction of APA was mediated by the adenosine A_1 receptor subtype.

Conclusions. Adenosine may modify RAS activity by induction of APA in the kidney. Thus, altered angiotensin repertory may have consequences in cardiac and renal injury in diabetes.

107) Dynactin complex and Dynein regulatory proteins are important for Murine Leukemia Virus (MLV) infection but not for Human immunodeficiency virus type-I (HIV-1) infection

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For viral infection to take place in a cell, a crucial requirement is the efficient transport of genetic material from the cell surface to the nucleus, it has been described that to reach their destiny, viruses utilize motor proteins that move unidirectional along microtubules or actin filaments, nevertheless the proteins involved in this process for MLV and HIV-1 are unknown. In previous work we demonstrate that the association of the incoming viral particle with the dynein complex is important for MLV infection. In this work we study the role of dynactin complex and dynein regulatory proteins in the infection of MLV and HIV-I. For this propose we generate cells were the dynactin complex and dynein regulatory proteins P50, P150, and NdeL1 were silenced. Then, we challenge these cells with MLV and HIV-I reporter viruses. We observe no significant effect on infection with HIV-I virus for P150 or NdeL1 knockdown cells, neither for P150 knockdown cells for MLV virus, whereas NdeL1 and P50 knockdown completely abolish MLV infection. Our results indicate that neither P150, nor NdeL1 play a role in the microtubule dependent retrograde transport of VIH-I. On the contrary, NdeL1 and P50 are important for MLV infection, were they could play an adaptor role in the association of the MLV to the dynein complex or directly to microtubules.

108) ALTERED SUBCELLULAR LOCALIZATION OF BRCA1 AND BARD1 IN BREAST CANCER TUMORS

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Breast cancer is the leading cause of cancer death among women worldwilde. Therefore, it represents an important health problem. In this regard, understanding the biology and molecular characteristics of breast cancer is important for the assessment of prognostic factors and planning treatment strategies. BRCA1 is a tumor suppressor gene, which mutations confer a high risk for breast cancer development. BRCA1 localizes at the nucleus where it accomplishes one of its mains functions: DNA repair. BRCA1 nuclear localization and retention is crucial for this function and is dependent on its interaction with BARD1. In this study, we analyzed the subcellular localization of BRCA1 and BARD1 proteins in 103 formalin-fixed and paraffin-embedded breast cancers tumors using immunofluorescence. Our results showed cytoplasmic BRCA1 localization in 51.4% of tumors with or without nuclear localization. In addition, 7.8% of tumors showed only nuclear localization and 40.8% presented absence of this protein. These results revealed that the vast majority of tumors present an altered BRCA1 localization which may compromise its function in DNA repair. Moreover, BRCA1 and BARD1 showed cytoplasmic co-localization in 51.4% of tumors and only 6.8% showed co-localization in the nucleus. These results indicate that only a small proportion of tumors may have a functional BRCA1/BARD1 heterodimer. In conclusion, since the majority of tumors in this study showed an altered expression or localization of BRCA1, our results contribute to the potential use of the expression of this protein as a marker for patient selection for PARP1 inhibitors and synthetic lethality treatment.

109) Galectin-3 activates pro-survival signaling pathways in fibroblasts but presents no obvious effect over cardiomyocytes

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Introduction. Galectin-3 is a □-galactoside-binding protein expressed in many organs, including the heart. Gal-3 is being evaluated as a biomarker for the development of heart failure (HF), but it is unknown if it has a direct role in disease progression. Cardiac remodeling is a process that precedes cardiac diseases including HF. It is characterized by cardiac hypertrophy, loss of contractility of cardiomyocytes and proliferation and activation of myofibroblasts. Finally, it can lead to cardiomyocyte cell death, a hallmark of HF. Here we asked if Gal-3 could influence cardiac remodeling either on cardiomyocytes or fibroblasts. **Methodology**. Primary cultures of cardiac myocytes and fibroblasts were stimulated with Gal3 10 ug/ml. Cell death was evaluated by Flow cytometry through PI incorporation and MTT assay. The activation of signaling pathways was evaluated by western blot. **Results**. The results obtained showed that Gal-3 has no effect on cardiomyocytes survival, at least at the times analyzed here. Also, Gal-3 did not change mitochondria metabolism or ATP production. In contrast, both, ERK and AKT phosphorylation were induced by Gal-3 on cardiac fibroblasts. **Conclusions.** Gal-3 is a biomarker for the development of HF, showing a straightforward relationship between plasma levels of Gal-3 and the impairment of cardiac function. Our results show for the first time the activation of pro-survival signaling pathways in fibroblasts and suggest that Gal-3 may have a role in the activation of fibroblasts into myofibroblasts without direct effects in cardiomyocytes.

110) Development of a novel and simple visible staining method for inexpensive DNA detection and quantification.

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DNA staining in gels has been historically carried out using silver staining and fluorescent dyes like ethidium bromide (EB) and SYBR Green I (SG I). Using fluorescence dyes allows recovery of the analyte and are more selective than silver, but requires instruments such as a transilluminator or fluorimeter to visualize the DNA. We designed a new and simple method that allows DNA visualization to the naked eye by a colored precipitate. It works soaking the acrylamide or agarose DNA gel in a 1,96µM SG I and 0,20 mM nitro blue tetrazolium (NBT) solution that, when exposed to light, produces a purple precipitate (possibly formazan). A calibration curve was made with a DNA standard to determine the detection limits. It is around 180 pg/band at 500 bp. The selectivity of the assay was determined using different biomolecules, such as $_{ds}$DNA, $_{ss}$RNA, proteins and lipopolysaccharides (LPS): only DNA was stained. Finally, DNA recovery tests were performed from an ampicillin resistant plasmid in an agarose gel stained with our method. A similar number of colonies were obtained compared with the traditional staining with SG I. This new methodology allows visualization of biomolecules to the “naked eye” without a transilluminator, which laboratories necessarily require nowadays; has potential benefits to research and industry laboratories due to fast detection, specificity, non-toxicity and for being inexpensive and adequate to field use.

111) Comparative analysis of flavonoids biosynthesis in three Fragaria species and their antioxidant and anti-platelet aggregation effects.

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Cardiovascular diseases (CVD) account for about 30% of world deaths, similar to the situation in our country. Several studies have shown that anti-platelet aggregation is effective in the prevention and treatment of CVD. During the last years there have been remarkable interests in plants and food which contain good antioxidant properties, in special berries due to their high content of potential bioactive compounds, such as polyphenols. Therefore, a comparative study of the polyphenolic composition and antioxidant properties of different strawberry fruit species (*Fragaria x ananassa* cv Chandler, *F. vesca* and *F. chiloensis ssp chiloensis* f. chiloensis and f. patagonica) was performed. The content of total phenolics (TP), flavonoids (TF) and anthocyanins (Ac) of whole fruit at the ripe stage was determined, as well as the free radical scavenging effect of the corresponding extracts by DPPH and FRAP assays. The highest TP, TF and Ac contents were found in *F. chiloensis* f. patagonica. *F. vesca* also had high TP content, while *F. x ananassa* also presented high TF and Ac contents following *F. chiloensis* f. patagonica. The highest free radical scavenging activity by DPPH assay was shown by *F. chiloensis* f. chiloensis, albeit its low content of TF and Ac. Meanwhile, *F. vesca* presented the highest antioxidant capacity measured by FRAP assay, probably explained by its high levels of TP and TF. On the other hand, the mRNA levels of genes encoding for key enzymes of the flavonoid biosynthesis pathway (CHS, CHI, F3H, ANS, DFR and UFGT) were also determined by qPCR. Results described high transcript levels in *F. x ananassa* for almost all genes analyzed, except for UFGT. In contrast, *F. vesca* had the lowest transcript levels in all genes evaluated, while *F. chiloensis* had an intermediate transcription levels. Additionally, aqueous extracts prepared from strawberries were analyzed for their anti-platelet aggregation activity and results showed that the aggregation of human platelets induced by ADP was inhibited by 1 mg/mL from all strawberry extracts. Extracts from *F. vesca* and *F. chiloensis* f. patagonica inhibit the aggregation of human platelets induced by ADP at 0.75 mg/mL. Nevertheless, strawberry extracts did not have inhibitory effect on collagen-induced platelet aggregation at a concentration of 1 mg/mL. As conclusion, the anti-platelet aggregation effect of different strawberry extracts could be due to their phenolic content and not to anthocyanins.

112) Regulation of ARK5 by LKB1 kinase

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Cancer cells evade cell death during tumor progression under metabolic stress. However, molecular mechanisms involved in cellular adaptation to this stress are still poorly understood. We and others have demonstrated that the serine/threonine kinase ARK5 is involved in the cellular response to metabolic stress. In addition, we have found that ARK5 expression increases in the nucleus, and is phosphorylated during metabolic stress. In this study, we have focused on LKB1-dependent regulation of ARK5 function, because ARK5 is known to be phosphorylated at threonine residue 211 by LKB1, which induces ARK5 kinase activity *in vitro*. For this aim, we have stable transduced Mouse Embryo Fibroblasts (MEFs) with lentiviral pLKO-shRNA scramble (control) or pLKO-shRNA LKB1 vector, and studied LKB1-dependent ARK5 expression, subcellular localization and cell survival under metabolic stress. Our data showed that LKB1 depletion increases ARK5 expression, suggesting a crosstalkbetween these two kinases. However, the nuclear localization of ARK5 and its function on cell survival were not affected. Consistent with a role of ARK5 in cell survival independently of its regulation by LKB1, overexpression of an ARK5 T211A mutant can promote cell survival under metabolic stress. Thus, we performed computational analysis of ARK5 protein sequence to predict other kinase-specific phosphorylation sites. Intriguingly, we found several sites predicted to be phosphorylated by JNK and p38. Future studies will investigate whether these kinases are involved in ARK5 phosphorylation and regulation of its cell survival function under metabolic stress.

113) Differential expression of microRNAs in breast cancer tumors associated to lymph node metastasis

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Breast cancer is the most common malignancy in women worldwide and it is responsible for the highest cancer-associated death rates. It is a heterogeneous disease and can be subclassified into several subtypes. Lymph nodes near to the primary breast tumor have an increased chance of developing a secondary tumor, representing one of the first signs of metastasis in breast cancer. The presence of axillary lymph node metastasis is a critical prognostic factor for the application of specific surgical and/or therapeutic strategies, and the amount of positive lymph nodes is known to have an inverse linear correlation with prognosis and survival. MicroRNAs are small non-coding RNAs that are involved in post-transcriptional regulation. Their expression has been frequently described to be altered in different cancer types, including breast cancer. MicroRNAs have emerged as candidate molecular biomarkers and novel therapeutic targets because of their stability, easy detection and ability to regulate a large set of genes that are involved in cancer growth and metastasis. In this work, we analyzed microarray based miRNA expression data to identify microRNAs differentially expressed in primary breast tumors with lymph node metastasis. We extracted total RNA from 31 fresh frozen breast tumors (Invasive Ductal Carcinomas) with different tumor grades (1 to 3). Patients did not receive neoadyuvant chemotherapy. Within the analyzed tumors, 16 were obtained from patients with a detectable lymph node metastasis. Microarray data analyzed using RankProd (R package) revealed 26 microRNAs downregulated in tumors associated with lymph node metastasis ($p<0.05$). Among the identified microRNAs 77% have been previously described to regulate early steps of metastasis in different cancer types, like members of the miR-200 family (miR-200c and miR-141), miR-205 and miR-30a. In addition we found other microRNAs not previously associated with metastasis, like miR-1202 and miR-199a-3p. Our results suggest that downregulation of an important proportion of the identified microRNAs, may lead to an over expression of their target genes in primary breast tumors, inducing a metastatic behavior of tumor cells, promoting invasion and colonization of lymph nodes. In this sense, the loss of expression of these microRNAs may serve as a new biomarker and/or indicator of prognosis in breast cancer patients.

114) MATERNAL OBESITY INDUCES ENDOPLASMIC RETICULUM STRESS AND AMINO ACID RESPONCE IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

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Maternal obesity (MO) is associated with fetal programming of adverse outcomes in the offspring, including insulin resistance (IR), diabetes mellitus and increased mortality due to cardiovascular diseases. Evidence shows that endoplasmic reticulum (ER) stress is involved in the development of IR in obese individuals. We evaluated transcriptional components of ER stress in human umbilical vein endothelial cells isolated from normal (N-HUVEC) and MO pregnancies (Ob-HUVEC) through lentiviral transduction of reporter gene constructs and qPCR. Relative luciferase expression was measured to assess activation of Amino acid deprivation response element (AARE), transcriptional response to ER stress (ERSE) and Activating transcription factor 6 (ATF6) response element, in presence of tunicamycin (ER stress activator) or TUDCA (ER stress inhibitor). Both, AARE and ERSE-dependent levels of luciferase detection were increased in Ob-HUVEC compared to N-HUVEC. However, TUDCA exposure was not associated to significant differences in the reporter gene activity of these response elements in both cell conditions. ATF6-dependent luciferase activity showed no differences between normal and MO derived cells, in absence or presence of TUDCA. All lentiviral constructs showed increased luciferase activity in presence of tunicamycin. In parallel qPCR experiments, we observed reduced mRNA levels of GADD34 and increased mRNA levels of BiP in Ob-HUVEC, which are related to AARE transcriptional signaling. These results suggest that MO increases the activation of AARE and ERSE response pathways of ER stress in HUVEC, which could generate transcriptional changes potentially relevant to development of MO dependent pathologies in the offspring´s umbilical endothelium.

115) DNA miss-recognition in mutated transcription factors across different cancer types

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We obtained a set of genes mutated in tumor exomes from 4327 patients affected with one of 19 different types of cancer from TCGA. Non-synonymous mutations were subjected to statistical analysis using MutSigCV obtaining 352 genes significantly mutated in cancer. 64 of these genes were identified as sequence-specific DNA binding proteins.

Mutations in transcription factors (TFs) can have three types of effects: loss of function, gain of function, and switch in DNA recognition. From the 64 DNA binding proteins mutated in cancer we identified TFs with crystallized structures under 2.2A˚ resolution and that had dsDNA bound. We then mapped the mutations from cancer patient exomes to these proteins leading to a dataset of 5 proteins and 16 mutations.Mutant models were created in Maestro, and minimized with FoldX. Resultant models were then subjected to a FoldX DNA specificity protocol. Logos of DNA specificities were created using 3DTF and PiDNA. In all cases we observed a switch in the DNA recognition motif of the selected proteins, which together with protein-DNA binding energy calculations suggested that cancer mutations in TFs switch their binding affinity for other DNA sequences.

In conclusion, we analyzed the full set of TFs significantly mutated in 19 types of cancer, proposing that some of these mutations cause a switch in the recognized DNA sequence. This switch of DNA specificity is a new type of effect of cancer mutations in TFs, having implications in their role in cancer genesis and progression.

116) LOCALIZATION OF TRANSCRIPTIONAL CO-REPRESSOR SKI ON SATELLITE DNA IN HUMAN MITOTIC CHROMOSOMES

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Ski is a transcriptional co-repressor for the TGFb and other signalling pathways. Protein levels are regulated through the cell cycle, with the highest levels found at G2/M. In mouse fibroblasts (MEFs and NIH3T3), we have recently found that Ski is located at the pericentromeric region of some mitotic chromosomes. In these regions, Ski is associated with Major Satellite (MaSat) and the absence of the protein in Ski -/- MEFs, resulted in decreased H3K9 trimethylation (H3K9me3), a critical modification for pericentromeric heterochromatin formation. In human cells, the localization and potential function of this protein on chromosomal structure have not been studied so far. Here, we addressed the localization of Ski in human chromosomes by performing indirect immunofluorescence (IFI) on metaphase chromosome spreads and by chromatin immuneprecipitation assays (ChIP-qPCR) in MCF-7 and MCF10A human cell lines. We found that Ski localized at human chromosomes, presenting a distinct dotted pattern at the pericentromeric region of several chromosomes. In order to identify satellite DNA occupied by Ski, we used RepeatMasker (UCSC) to design chromosome-specific primers at repetitive regions on the genome, specifically for Beta Satellite (BSR) and Human Satellite II (HSatII) repetitive regions. ChIP-qPCR assays showed a significant enrichment of Ski occupancy of BSR at chr 15, and HSatII at chr 22. Considering previous results in mouse chromosomes, this data suggest a potential function of Ski on pericentromeric heterochromatin maintenance in human chromosomes, which could have a significant impact on chromosome segregation and genome stability.

117) CCT type II chaperonin promotes *in vitro* γ-tubulin self-aggregation. An interesting new function related to the protein misfolding cell response.

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Although it has clearly been demonstrated that the chaperonin CCT binds non-native proteins to assist their folding, here we show experimental evidence that it can also promote the in vitro and in vivo off-pathway aggregation of γ-tubulin, in a process that is probably related with the cell response against protein aggregation toxicity. We initially set out to characterize the γ-tubulin-CCT binding process using the canonical assay for aggregation inhibition. In theory, CCT should produce an inhibitory effect over tubulin aggregation, but surprinsingly, addition of CCT generated an aggregation-promoting effect over γ-tubulin. The free energy for this process was 4.34 kcal/mol. A decrease of critical concentration value for the aggregation was observed, indicating that this process is facilitated by a nucleation effect induced by CCT interaction. This occurs even at low CCT:γ-tubulin ratio. The scarce presence of CCT into the aggregates was confirmed by electron microscopy and co-sedimentation analysis. Addition of ATP boosts the aggregation process. In the absence of ATP a small population of CCT-γ-tubulin complex remains soluble. Using different electron-microscopy techniques combined with image processing we characterized the structure of this particle, and by sequence correlated-mutations-analysis we identified the CCT-γ-tubulin interaction regions. To confirm experimentally these regions we constructed γ-tubulin mutants and assayed their binding to CCT. The results showed a decrease in the off-pathway aggregation-promotion, thus confirming the specificity of this phenomenon. By injecting γ-tubulin protein into zebra-fish early embryos we observed that γ-tubulin mutants form smaller intracellular aggregates, suggesting that this effect has in vivo relevance.

118) Effects of handling stress on the expression of genes involved in energetic metabolism in red-cusk eel (*Genypterus chilensis*).

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Introduction: Chilean aquaculture is mainly focused on salmon production, evidencing a clear necessity for diversification. The red-cusk eel has been proposed as one of the candidate to achieve this goal. However, this species in captivity is susceptible to stress, showing low growth rates, which could de due to alteration in energetic metabolism in skeletal muscle. Thus, the aim of this research is to study the expression in muscle of genes involved in energetic metabolism in the red-cusk eel under handling stress.**Methodology:** Through *in vivo* approached we assessed the effects of handling stress on gene expression in muscle of molecules involved in energetic metabolism such as: 5' AMP-activated protein kinase *(ampk*), peroxisome proliferator-activated receptor gamma (*pgc-1a*), sirtuin 3 (*sirt3*), thioredoxin reductase (*tr3*). **Results and Conclusions:** Stressed fish showed a significant increase in mRNA contents of *pgc-1a*, in contrast with *sirt3, tr3*, and *ampk* which showed no significant changes. Amounts of carbonylated proteins and lipid peroxidation also did not show changes. Our results suggest that handling stress may be affecting energetic metabolism via induction of mitochondrial biogenesis

119) Role of anticancer drugs that promote SG assembly on HIV-1 replication

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In the last 30 years, drugs have been developed to target specific processes of the HIV-1 replication cycle, which have been essential in maintaining life quality of patients. However, HIV-1's ability to develop resistance to most current ARTs urges us to identify new therapeutic targets. Following infection, the host cell initiates an anti-viral immune response to create an inhospitable environment for viral replication, causing both the shut-off of protein synthesis and stress granules (SGs) assembly. SGs are translationally silent ribonucleoproteins (RNPs) and serve as storage sites for mRNAs and proteins. Studies have revealed that HIV-1 Gag protein suppresses SG assembly, but when not possible, it not only lifts the SG blockade, but also results in impaired virus production and infectivity. Given that anti-carcinogenic drugs (ACDs) can induce the assembly of SGs, we hypothesize a potential effect of ACDs on HIV-1 replication in the short-term. To test this hypothesis, HeLa and U2OS cell lines were exposed to three different ACDs, Doxorrubicin (DOXO), Etoposide (ETO) and Vorinostat (SAHA) in the presence of HIV-1 provirus plasmid. Immunofluorescence was used to observe the presence of SGs, and WB/RT-qPCR to analyze the viral replication process. Sodium arsenite and Puromycin was added as a positive control for SGs assembly. Our results suggest that ACDs modulates HIV-1 replication on cells that have SGs assembly, which indicates a potential use of ACDs as new and effective antiretrovirals.

120) Peptide extracts present in sea cucumber, *Athyonidium chilensis* (Semper, 1868), with potential anticancer activity

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Introduction: In the last decades many biological active compounds have been isolated and characterized from several marine species. These compounds, mainly extracted from sponges, mollusks, bryozoans, tunicates and echinoderms have shown a wide spectrum of biological properties. A great number of bioactive molecules have been obtained from the sea cucumber, which belongs to the class Holothuroidea. Particularly, peptides isolated from the sea cucumber aroused interest for their potential biomedical application. Despite the wide diversity of species found in Chile, there are no studies that use these peptides for the treatment of gastric cancer.

Methods: In this study, anticancer activity of three peptide fractions (FP1, FP2 and FP3) obtained by enzymatic hydrolysis of protein extract of sea cucumber Athyonidium chilensis were assayed on AGS gastric cancer, using the MTT method for cell viability.

Results: FP2 fraction shown a high activity, inhibiting the growth of gastric cancer cells at a concentration of 100µg/ml. Similar results were obtained with the FP3 fraction.

Conclusions: Peptidic extracts from sea cucumbers showed high anticancer activity against AGS cells, which could be an interesting opportunity for the gastric cancer treatment using marine natural compounds, abundant in Chile.

121) Transcriptomic analysis of common carp (Cyprinus carpio) in seasonal acclimatization process.

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The response of organisms to environmental fluctuations is complex and involves multiple factors that are mostly unknown. It is known that this response is not random, but it involves reprogramming at the level of gene expression, modeling the individual response. For this reason we have evaluated these responses to changes in the environment using RNA-Seq during seasonal acclimatization of common carp (Cyprinus carpio). In this work we have obtained transcriptomic information using Illumina sequencing technology Miseq. They were obtained after the sequencing of carp liver. For summer and winter, 38,012,838 and 34,620,343 RNA readings where generated respectively. After a filtering process, subsequent readings and de novo assembly using the software Trinity. 250,000 contig where obtained by creating a reference transcriptome of a N50=1279pb. The database generated contribute useful information for understanding the molecular mechanisms that are involved in the process of seasonal acclimatization of common carp, further studies are needed to better understand this process.

122) Virulence and Pathogenic Effect of Influenza A(H1N1)pdm09 Genetic Variants with Different Plaque Phenotypes

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The pandemic influenza A strain, H1N1pdm09 originated in swine resulting from a triple genetic reassortment between swine, human and avian viruses. Genetic variations within a virus population can play a cooperative role in the replicative capability and the outcome of the infection. We identified that the prototypic H1N1pdm09 strain, A/Netherlands/602/2009 had a mixed plaque phenotype. Early passages of this virus had a lethal phenotype in mice at a dose of $1.58X10^4$ pfu, additional passages (>4) *in vitro* resulted in a virus with reduced virulence. We isolated two variants of this strain, one with large (BP) and one with small plaque (SP) phenotypes in MDCKs. As compared to the BP virus, the SP isolate demonstrated a comparatively faster replication-kinetic in the first 24 hrs of infection in MDCKs and produced greater weight loss in mice, at levels comparable to the low passage WT stocks. Sequencing of these variants revealed mutations in the Hemagglutinin (HA-E172G) and the Nucleoprotein (NP-R437G) of the BP virus, as compared to the WT strain. Moreover, the SP virus had mutations in polymerase basic-2 (PB2-S155C) and in the Neuraminidase (NA-I407V) proteins. We speculate that these mutations are responsible for the phenotype differences and produce changes in the replicative capacity of these viruses, which might have a direct effect in their virulence. Recombinant viruses are under preparation to characterize these viral phenotypes by reverse genetics and minigenome assays. Understanding the role of each observed mutation can potentially aid in predicting the pathogenic potential of clinical isolates from ill patients.

123) Accuracy assessment of an automated MMGBSA-based protocol to estimate binding free energies on the PDBbind data set

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In the drug discovery process, one of the more challenging issues is the calculation of the free binding energies (DG) of a large set of small molecules to molecular targets of clinical interest. In this regard, implicit solvation methods coupled with MM force fields (e.g., MM-GBSA, MM-PBSA) have grown in popularity in the recent years for its low computational cost; however, there is still no general consensus on an efficient protocol that implements such methodologies to determine affinity energies in good agreement with experimental data. We have implemented an automated protocol to estimate the DG values using the MM-GBSA approach on minimized protein-ligand complexes, including explicit energetic terms related to the protein and ligand deformation upon binding, and taking advantage of recently reported advancements that contribute to a fast and robust scoring function.

As preliminary results, we assessed the performance of the implemented protocol against a set of protein kinase (CDK2, Aurora A and P38) inhibitors, where the correlation between the predicted binding energies and the experimental data is acceptable in most cases (R^2 up to 0.7). Here we present an extensive evaluation of the protocol carried out against the PDBbind core set, which provides a curated set of over 200 protein-ligand complexes with affinity data deposited in Protein Data Bank (PDB). So far, the DG estimation protocol has presented an acceptable correlation with experimental data, demonstrating its potential utility in the fields of computational biology and drug-design.

124) SR-SASA: a new tool for indentifying and quantifying interaction surfaces in biological molecules based on the buried solvent accessible surface area

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Calculating interactions between molecules is a crucial step in many types of biological and biotechnological applications, from drug design to directed mutagenesis, where identifying regions of interest in the interface between biological macromolecules can give useful insight in which atoms or residues the experiments or other analysis should be focused on. Here we present the development of a computer tool called SR-SASA, which identifies and quantifies the area of the interacting surfaces in macromolecules at the atomic and residue level. It takes as an input a PDB file and, optionally, a definition of objects (chains, aminoacids/ nucleotides or atoms). The interacting surface for each pair of given objects is defined as the atoms that have their solvent accessible surface area (SASA) in one object buried by the atoms of the other. Our modified Shrake-Rupley algorithm makes this tool capable of identifying and quantifying the interaction area between the atoms in the SASA of each object, which is a unique feature among all existing tools. Our application was benchmarked against NACCESS, a widely used computer program for SASA calculations using a large and non-redundant set of protein-DNA complexes. We obtained an average difference of 0.098 Å² in the calculation of per atom SASA, with a standard deviation of 0.152 Å². This computer software is freely available upon request for the LINUX operating system.

125) Standardization of Comet Assay for the Evaluation of Genotoxic Damage caused to *COLO320 Carcinoma Cell Line*

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Genotoxic stress is one of the most employed therapy methods for the treatment of illnesses such as cancer. The comet assay in alkaline conditions allows the analysis of the damage induced by genotoxic agents in cell cultures and harm identification in the genomic DNA, both in single-stranded DNA as well as in alkali sensitive areas. Our purpose was to evaluate the response of *COLO320* carcinoma cell line to genotoxic stress with different concentrations of Etoposide and Doxorubicin during 6 hours. A modified protocol of comet assay adapted from Singh et al.,1988 was employed; *Low Melting Point* agarose (LMP) was employed to make the intermediate layer, then *Normal Melting Point* agarose (NMP) for the first and third layer. Subsequently, an alkaline lysis for 24 hours was performed, followed by denaturation for 20 min in electrophoresis buffer. Next, an electrophoresis (cold) at pH 10, 300 mA for 25 min. was carried out. Afterwards, washings in a Tris pH 7.5 buffer were performed. Finally, samples were stained with GelRed 3x and they were analyzed in Epifluorescence microscope. Results show different damage levels in the DNA. In the case of Etoposide treatment, it was seen that 50 mM concentrations already generate comet tails that increase in number and length when concentrations achieve 100 mM, and they increase even more for 150 mM concentrations. These particular results show that DNA's extent of damage is directly proportional to the concentration of the compound used. In the case of treatment with Doxorubicin the results were similar.

126) Development of a new model for predicting DNA flexibility from sequence.

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Introduction: Nucleotide sequence determines DNA structural dynamics and thereby its flexibility, affecting protein-DNA recognition in biological processes such as gene expression or nucleosome formation. In this work we have used data from experimental structures to generate a new computational model for predicting regions of higher/lower flexibility from a primary DNA sequence. **Methodology:** 87 free duplex DNA structures from the Protein Data Bank (PDB) were used to calculate the structural dispersion (RMSD) for every possible dinucleotide superposition. Based on these values, a computer program was implemented, which assigns an expected RMSD value per nucleotide in the DNA sequence that constitutes a flexibility profile. **Results:** The results obtained based on six different angle models previously published were compared to the predictions of flexibility using our model for five different test sequences. Our new di-nucleotide model shows a high similarity with the tri-nucleotide model based on nucleosome positioning, which is the more robust and accepted model among all tested. **Conclusions:** These results show that this new model of flexibility based on less information (di-nucleotides), is able to predict bending sites calculated using more information (tri-nucleotide). This model of flexibility could be implemented in the 3D modeling pipeline of duplex DNA to facilitate the recognition of regions with more/less flexibility to be modeled with greater/lesser degrees of freedom according to this information.

127) *Arabidopsis thaliana* **growth and salinity tolerance are induced by** *Burkholderia phytofirmans* **PsJN through bacterial emission of a blend of volatile signals**.

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Environmental stress limits the growth of plant crops worldwide. However, interaction with certain plant growth promoting rhizobacteria (PGPR) can improve the rate and extent of plant growth in the presence of stress and environmental challenge. *Burkholderia phytofirmans* PsJN, a well-studied PGPR, is reported to produce hormone phytostimulation of different plant hosts. We have shown that inoculation with strain PsJN is able to enhance salinity tolerance of *Arabidopsis thaliana*, but the bacterial functions involved in this effect are yet unclear. Furthermore, this effect could result from bacterial activity in contact with plant tissues, and/or from long range signaling among partners, without the need of actual root colonization. The aim of this work was to assess the role of bacterial volatile organic compounds emissions from *B. phytofirmans* on plant tolerance to saline stress, comparing growth and response to salinity in *A. thaliana* directly inoculated *in vitro* with strain PsJN, and in plants exposed to volatile emissions from the bacterium, when grown in a separate container. Additionally, the contribution of specific bacterial functions, putatively involved in plant stress alleviation, was assessed using PsJN mutant derivative strains. Growth, stress response, and sodium homeostasis were analyzed for plants under different experimental treatments. Our results indicate that PsJN-induced salinity tolerance in Arabidopsis is mainly dependent on emission of a blend of volatile long-chain alcohols and ketones, which is only effective during a specific stage of plant development, while *in situ* ACC-deamination and IAA homeostasis have only a minor role on the overall effect of *B. phytofirmans*.

128) Gemcitabine-resistant gallbladder cancer cells have epithelial-to-mesenchymal features and overexpression of ABCC2 transporter

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Introduction: Gallbladder cancer (GBC) is an endemic disease in Chile, with a dismal prognosis and an inherent resistance to gemcitabine, the current standard chemotherapeutic drug. In this study we selected chemoresistant TGBC1 GBC cells by incubating them in the presence of gemcitabine for an extended period of time. Transcriptomic analysis was performed on wild type (WT) and resistant (R) clones to identify differential gene expression. **Methology**: GBC cell line TGBC1 was cultured in presence of gemcitabine during 4 months until it reached a difference of 50-fold less sensitive than WT cell line, analyzed by the IC50 assay. Transcriptomic analysis was performed with Affymetrix's Human Transcriptome Array 2.0. Immunocytochemistry assay (ICC) was performed for epithelial and mesenchymal markers. **Results**: Resistant TGBC1 clone has 55-fold increase in IC50 compared with its WT cell line. Transcriptomic analysis of the resistant clone reveals an increase in mesenchymal markers (VIM, CNN1, TAGLN, ZEB1) and a decrease in epithelial genes (KRT19, KRT7, EPCAM, MUC1). ABCC2, member of the ABC transporter family, was found to be overexpressed at mRNA and protein level. ICC analysis validated the transcriptomic data and revealed a mesenchymal-like population that was already present in the WT cell line at low frequency (<5%). **Conclusions**: TGBC1 Gemcitabine-resistant GBC cell line posses epithelial-to-mesenchymal transition features. ABCC2 transporter may in part contribute to chemoresistance, however, future studies are needed to address this hypothesis.

129) A-to-I transcriptome editing mediated by ADAR1 in breast cancer.

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The double stranded RNA-specific adenosine deaminase (ADAR1) catalyzes the deamination of adenosine in non-coding RNAs, pre-mRNAs and matures mRNAs resulting in important changes in stability, function and expression of its targets. Editing unbalance has been demonstrated in several diseases, including cancer, where increased editing levels are strongly correlated with the progression of the disease. However, the expression and activity of ADAR1 in breast cancer is not well characterized. Gene expression data from The Cancer Genome Atlas (TCGA) cohort, which includes 1214 breast cancer samples, was analyzed implementing a novel strategy to call single nucleotide changes in the RNA, in order to evaluate the editing changes present in these patients. In addition, the expression levels of ADAR1 and the editing levels of ADAR1 targets were measure using RT-qPCR and RESSqPCR in three breast cancer cell lines (MCF10A, MCF7 and ZR-751). The analysis of expression data from TCGA showed a significant overexpression of ADAR1 in tumors compared to normal samples. These changes also correlated with the editing and expression levels of ADAR1 in the breast cancer cell lines analyzed. In addition, the bioinformatics analysis revealed a significant enrichment of A>G transition in the tumor compared to normal samples, showing significant editing changes in mRNAs associated to the DNA replication and cell cycle process. Impending work is needed to elucidate the function of the editing changes mediated by ADAR1 and their relation with the breast cancer progression.

130) EFFECT OF NORDIHYDROGUAIARETIC ACID ON GLUCOSE TRANSPORT AND ACCUMULATION IN HUMAN LEUKEMIC CELL LINES.

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Polyphenols are natural products with antioxidant and antiproliferative properties. Since cancer cells have a high dependence on glucose metabolism, to hamper the growth of neoplastic cells, a proposed strategy is to generate an energy-deprived state by the pharmacological inhibition of glucose uptake. Nordihydroguaiaretic acid (NDGA) is a polyphenol extracted from the bush L. tridentata. Since there is no evidence of the effect of NDGA on hexose metabolism in neoplastic cells, we study the effect of NDGA on glucose transport and accumulation on two human leukemic cell lines: U-937 and HL-60. NDGA behaves as a competitive blocker of glucose uptake in HL-60 cells under zero-trans entry assays, but as a mixed noncompetitive blocker in U-937 cells, suggesting that NDGA interacts with the transporter's external ligand binding site. By other hand, accumulation experiments show that NDGA is a non-competitive blocker of metabolic glucose trapping in both cell lines. Under cis-infinite conditions in human erythrocytes, NDGA behaved as a competitive blocker of glucose exit, displacing glucose from the external binding site. Besides, CCB displacement assays in red blood cell membranes showed that NDGA interacts directly with GLUT1. These results suggest that NDGA acts as a direct inhibitor of glucose uptake mediated by GLUT1 in U-937 and HL-60 leukemic cell lines and thereby affects substrate accumulation in these cells.

131) Regulation of gene expression of the nuclear-encoded mitochondrial protein HIG2A by hypoxia and cellular metabolism

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Introduction: Respiratory supercomplexes are dynamic assemblies of complex I, III and IV within the inner mitochondrial membrane, allowing the adjustment of respiratory control to cell´s energy demands. Assembly factors are then required for the configuration and stability of supercomplexes. In this regard, HIG2A has been recently described and it was shown that HIG2A knocking down impaired supercomplex formation. However, how HIG2A is regulated at the level of gene expression is not known. Our current objective is to study the regulation of the *higd2a* gene that encode for HIG2A.

Methodology: We performed bioinformatics analyses of HIG2A; *higd2a* gene expression by qRT-PCR; and reporter assays. Tests were performed in cell lines and C57BL/6 mice.

Results: HIG2A has a hypoxia-inducible domain and *higd2a* expression was increased significantly in C2C12 cells after hypoxia exposure. Promoter region of *higd2a* possesses binding sites for PPAR-α and E2F-1 transcription factors. We modulated chemically PPAR-α and E2F-1 in C2C12 and HEK293 cells and this induced significant changes on *higd2a* expression. In mice, *higd2a* expression showed differential tissue expression, which was higher in bone marrow than in liver and spleen. Furthermore, we observed that mice injected with quercetin, a modulator of energetic metabolism, displayed a tissue-differential expression of *higd2a*. In spleen and bone marrow, *higd2a* expression increased significantly, while in liver decreased significantly as compared with control mice.

Conclusions: Alterations in cellular metabolism lead to changes in *higd2a* gene expression. HIG2A protein might function as a regulator of respiratory supercomplexes in response to changes in cellular metabolism.

132) A synthetic red-light toggle switch to control gene expression in *Neurospora crassa*.

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The fungus *Neurospora crassa* is a model organism for circadian studies, however it is blind to red light stimulation. Phytochromes are red-light photoreceptors, with the ability of photo conversion between the active (660 nm) and inactive (740 nm) state in response to red light. Synthetic biology based strategies have used phytochromes to develop new optogenetic switches, allowing specific control of gene expression. With the aim of implementing an orthogonal optogenetic system based on red-light induction in *N. crassa,* we developed a synthetic red-light toggle switch to control gene expression. The genetics constructions were designed *in silico*, assembled *in vivo* using yeast recombinational cloning and integrated in the *N. crassa* genome by homologous recombination. Initially, we used the yeast GAL4 transcription factor with codon-optimization for *N. crassa* as a positive control for orthogonal regulation of gene expression. The results showed 5 times fold induction of a luciferase reporter gene under GAL4-UAS promoter control. Based on these results, we designed the red-light system using PhyB and PIF6 from *A. thaliana* expressed as chimeric proteins using GAL4 activation domain (AD) and GAL4 DNA binding domain (DBD) from yeast, respectively. The synthetic system was fully codon optimized for *N. crassa* and upon red-light stimulation, PhyB-GAL4-AD and PIF6-GAL4-DBD interaction activated the expression of the luciferase reporter gene under the GAL4-UAS promoter control. In conclusion, a synthetic red-light toggle switch was implemented in *N. crassa*, showing potential for orthogonal perturbation of transcriptional networks and heterologous protein expression.

133) Insulin requires A_{2B} adenosine receptors activation to restore fetoplacental human endothelial function in late-onset preeclampsia.

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Preeclampsia (PE) associates with reduced placental vasodilation, endothelial nitric oxide synthase (eNOS) activity and L-arginine plasma level in human fetoplacental circulation. Adenosine and insulin cause vasodilation of the human umbilical vein involving adenosine receptors (ARs) activation in uncomplicated pregnancies. However, under pathological conditions such as PE, plasma adenosine level increases in umbilical vein and also the A_{2B} adenosine receptors (A_{2B}AR) expression in human umbilical vein endothelial cells (HUVECs). Elevated placenta adenosine level not only contributes to the pathogenesis of PE, but A_{2B}AR could have a role in the placental development. **Objectives.** The aim of this study was to determine the role of A_{2B}AR in the endothelial L-arginine/NO signalling pathway and vascular reactivity in response to insulin in HUVECs from late onset preeclampsia (LOPE). **Methods**. Vascular reactivity to insulin (0.1-1000 nmol/L, 5 min) was measured in KCl-preconstricted human umbilical vein rings (wire myography) from normal and LOPE pregnancies in the absence or presence of adenosine (1 mmol/L) and/or the A_{2B}AR antagonist (MRS-1754, 30 nmol/L). The protein level of total eNOS and L-citrulline formation were determined by Western blot and high performance liquid chromatography (HPLC), respectively, in the absence or presence of insulin (1 nmol/L) and/or MRS-1754 in HUVECs from normal and LOPE pregnancies. L-Arginine transport (100 µmol/L, 3 µCi/mL L-[^{3}H]arginine, 1 min, 37°C) was measured in the absence or presence of insulin and/or A_{2B}AR agonist (NECA, 1 µmol/mL) and MRS-1754 in HUVECs. **Results**.LOPE associates with reduced insulin-mediated umbilical vein ring relaxation compared with normal pregnancies, which was improved by adenosine, an effect abolished in the presence of adenosine + MRS-1754. LOPE increased total eNOS expression and activity compared with normal pregnacies, and the A_{2B}AR antagonist blocked these effects of LOPE. LOPE increased hCAT-1-mediated L-arginine transport in HUVECs, an effect unaltered by the A_{2B}AR agonist alone, but blocked by the A_{2B}AR antagonist in the presence of insulin. **Conclusion.** HUVECs from LOPE exhibit impairment of A_{2B}AR-mediated L-arginine/NO signalling pathway. A_{2B}AR activation in response to insulin is required to restore the relaxation and endothelial function in LOPE pregnancies.

134) Degradation of a knotted protein by the ATP dependent protease ClpXP of *Escherichia coli.*

San Martín, Alvaro[1]., Molina, José[1]., Baez, Mauricio[1]., [1]Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad De Chile. (Sponsored by Proyecto Fondecyt Chile, Código: 1151274: Folding And Degradation Of Proteins With Knotted Topologies: Implications For Folding Of Proteins And Unfolding And Translocation Mechanism Of ATP-dependent Proteases (ClpXP).)

Unlike other polymers, proteins avoids the formation of knots when they are compacted during folding, in fact only 0,5% of known protein structures are knotted. The reason why there are so few knotted proteins has not yet been elucidated. It has been hypothesized that knotted proteins could obstruct biological processes that requires protein translocation through narrow pores, like protein degradation by ATP dependent proteases. Here, we explore that possibility by characterizing the degradation kinetics of the knotted protein MJ0366 from *M. jannaschii* by the ATP dependent protease ClpXP of *E. coli*. To achieve this a knotted fusion protein was constructed by the addition of the green fluorescent protein (GFP) to the N-terminus of MJ0366 and a degradation signal (ssrA-tag) to the C-terminus (GFP-MJ0366-ssrA). The *in vitro* degradation assay of GFP-MJ0366-ssrA did not show a significant decrease of GFP fluorescence over time. Furthermore, the degradation of GFP-ssrA by ClpXP was inhibited in presence of GFP-MJ0366-ssrA, indicating that the knotted fusion protein is recognized but not degraded by the ClpXP protease. These results are the first *in vitro* evidence of how the presence of knots in proteins can be unfavorable for some biological processes. Currently we are characterizing the kinetics of degradation of knotted proteins with different structures to determine if the knotted topology is a general feature incompatible with protein degradation mediated by the ATP dependent proteases.

135) miR-335-5p is a potential suppressor of metastasis and invasion in gastric cancer

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Introduction: miR-335-5p has been previously identified with tumor suppressor features, and low expression has been linked to cancer development. The aim of our study was to evaluate the potential tumor suppressor role of miR-335-5p in metastasis and invasion of GC. **Methods:** We analyzed the expression of miR-335-5p in 23 hispanic/amerindian advanced GC tissues relative to their paired adjacent non tumor tissues (NAT) and 9 GC cell lines by quantitative real-time PCR. Clinico-pathological correlations were performed. Cell migration, invasion and clonogenic assay were performed in cells transfected with miR-335-5p mimics to induce overexpression and with miR-335-5p inhibitors to produce underexpression. **Results:** Analyzing 23 samples we found a reduced expression of miR-335-5p in GC tissues when compared to NAT ($p < 0.005$). Multivariate analysis revealed that miR-335-5p expression is associated to lymph node metastasis ($p < 0.005$) and depth of tumor invasion ($p < 0.005$), independently. In addition, patients with low expression level of miR-335-5p have five times higher risk of developing lymph node metastasis than patients with high expression level of miR-335-5p, (OR= 5.000, $p < 0.005$). Likewise, low expression level of miR-335-5p was a significant independent prognostic factor for reduced median overall survival (HR= 3.960, $p < 0.005$). Moreover, decreased levels of miR-335-5p expression were found in nine human GC cell lines compared with NAT ($p < 0.005$). In AGS cells, miR-335-5p overexpression was correlated with a reduction in the migration ($p < 0.005$), invasion ($p < 0.005$) and clonogenic capacities ($p < 0.005$). Conversely, we observed an increase in the migration ($p < 0.005$), invasion ($p < 0.005$) and clonogenic capacities ($p < 0.005$) in knockdown AGS cells group in comparison with the control group ($p < 0.005$). **Conclusions:** This study showed clinical significance of miR-335-5p expression in a cohort of patients, reporting that its low expression is significantly associated to lymph node metastasis and tumor invasion. Besides, low miR-335-5p expression was correlated with poor survival of study group. *In vitro*, expression level of miR-335-5p was downregulated in gastric cancer cells lines and their upregulation decreases migration, invasion and clonogenic capacities. Taken together our results provide new evidence of the role of miR-335-5p in tumor aggressiveness processes such as metastasis and tumor invasion.

136) PDGF-BB induces mitochondrial fragmentation and degradation during VSMC dedifferentiation

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Dedifferentiation of vascular smooth muscle cells (VSMCs) from a contractile to a proliferative/migratory phenotype, play a key role in development of vascular pathologies. In these cells, mitochondrial number and activity could have an important role in maintaining the contractile phenotype. Here, we evaluate the effect of platelet-derived growth factor (PDGF-BB), a potent proliferative/migratory phenotype inductor, on mitochondrial autophagy and activity during the phenotypic change. VSMC A7r5 were treated with PDGF-BB (10 nM) for 0-24 h. Mitochondrial morphology was assessed by confocal microscopy in A7r5 stained with Mitotracker Orange, mitochondrial potential (Δψm) was evaluated with JC-1 and flow cytometry, and ATP content by luciferase assay. PDGF-BB induced mitochondrial fragmentation and decreased Δψm without affecting total ATP content. PDGF-BB induced autophagy as detected by LC3-II increase and p62 decrease. Colocalization of mitochondria with autophagosomes was increased with PDGF-BB treatment. PDGF-BB also induced a reduction of mitochondrial content, as evaluated by mitochondrial DNA content. All of these changes precede the PDGF-BB-induced proliferation and migration, assessed by [^{3}H]-thymidine incorporation and wound healing assay. Thus, the understanding of the process that involves the phenotypic changes in VSMCs may lead to development new strategies in treatment of vasculopathies.

137) THE HYDROPHOBIC INTERACTIONS STABILIZE THE BINDING OF DKTX TOXIN AND THE PAIN RECEPTOR TRPV1.

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In nature, animals and plants use painful toxins as defensive mechanism against predators or competitors. Most of the toxins act as irritants or cause pain by interacting with functional domains of ion channels. A target of these painful toxins is the transient receptor potential vanilloid 1 (TRPV1) which is a cation-selective ion channel expressed in primary sensory neurons. TRPV1 is an pain receptor modulated by multiple noxious stimuli as high temperature (>42º), low pH (< 6) or irritants compounds. The Double Knot toxin (DkTx) from Ornithoctonus huwena spider, is a polypeptide which interacts with the extracellular surface of TRPV1 disturbing the selectivity filter and driving the activation of the channel. Even when interaction surface between TRPV1 and DkTx has been identified by site directed mutagenesis and the cryo-EM structure, the structure of DkTx and its binding mechanism remain unknown. In order to understand the atomic interactions involved in DkTx binding, we build a homology model of DkTx toxin which was subsequently docked to the TRPV1 putative binding surface structure to then performing molecular dynamics simulations. Our results suggest that each domain of DkTx toxin independently interact with a single subunit of the TRPV1 tetramer and these interaction network is strongly stabilized by hydrophobic residues in both interacting surfaces.

138) Analysis of the transcriptional expression in response to an abiotic natural stress (salinity) in a freshwater fish (*Cyprinus carpio*).

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(Sponsored by (Sponsored By CONICYT/FONDAP/15110027))

In many studies, it has been established that different environmental variables influence the organisms. Salinity is an environmental input widely studied in teleost fish because a delicate regulation is needed to maintain homeostasis, a process called osmoregulation, and involves changes in the behavior, physiological, cellular and / or molecular levels.

The Budi Lake (IX Region, Chile) is a coastal salt lake in which lives naturally carp (*Cyprinus carpio*). Consequently, carp emerge as suitable biological model to study the molecular mechanisms implemented by a freshwater fish to adapt at this stressful condition.

In this work, we evaluated through RT-qPCR the transcriptional expression of different genes involved in osmoregulation such as channels, ionic transporters, hormonal receptors, and molecular factors involved in ribosomal biogenesis. Our results revealed a significant up regulation of genes involved in osmoregulation and epigenetic control during the high salinity season. Conversely, ribosome biogenesis was significantly down regulated (up to 50%) in response to high salinity conditions. In conclusion, our results demonstrate that carp adaptive capacity to salinity is based on a delicate reprogramming of specific target genes. Consequently, the present study constitutes a first attempt to analyze the molecular strategies that displays a freshwater fish against salinity stress.

139) Functional inference of proteins involved in valine biosynthesis and their use to define bacterial species: Insight from a novel Antarctic bacteria

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The valine biosynthetic pathway is composed of five enzymes that catalyze the conversion of 2 molecules of pyruvate to either valine or isoleucine, implying that these proteins are not strict in their final product specificity. In this work, we explored each protein family of the enzymes involved in the valine biosynthesis pathway to determine differences at the sequence level between those enzymes that conduct to valine from those that conduct to isoleucine. This in turn, allows us to define the phylogenetic clades to which an organism belongs based on sequence similarity to enzymes from known organisms. Using known enzymes from the valine biosynthesis pathway we performed BLASTp searches against nr database and complete and reference proteomes in Uniprot. Then, we carried out all against all comparisons for each set of homologous proteins and visualized the comparisons as sequence similarity networks, in which the clusters contain proteins with the same product specificity and at the same time from evolutionarily related species. We demonstrate here, the potential use of metabolic enzymes to define bacterial species, and the results were contrasted to standard methods for species identification: phylogenetic trees using 16S rRNA sequence and Average Nucleotide Identity (ANI). We then applied this methodology to functionally characterize and phylogenetically identify a novel valine producing bacteria isolated from the Antarctic territory, which Aminotec had earlier discovered to be a good secretor of valine and which we have recently sequenced and assembled.

140) Role of glucose during fruit ripening of *Vitis vinifera.*

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Grapevine fruit development can be divided into three stages: the formation stage, the lag phase, and the ripening stage; during which occurs physiological and biochemical changes that allow the accumulation of different solutes and cell differentiation.

During the ripening stage, several genetic, morphological and physiological changes take place, allowing the completion of the berry development. Among these we can find the increase of sugar concentration (especially glucose and fructose), the increase of anthocyanin concentration and size, the decrease of turgor and acidity, and hormonal variations.

Despite there is widespread knowledge about berry grape development, the nature of the signal that initiates and facilitates the coordination of the ripening process still remains unknown.

In order to determine which is the signal that initiates and regulates the ripening in grape berry, this work is mainly focused on understanding the role of glucose and fructose during berry development, due to the increment of these molecules in the beginning of this stage.

Genetic and phenotypic analysis was used in order to determine the degree of ripening of the berry. The results of this work allowed us to demonstrate the importance of glucose in the regulation of the beginning of ripening, because pre-veraison berries treated exogenously with glucose matured 20 days earlier than fruit treated with water. Moreover, sugar treatment of cell cultures of grape showed that the expression of the *MybA1* gene, which is essential for anthocyanin biosynthesis, is induced in response to glucose.

141) Study of STIM1-Orai1 in cardiomyocyte autophagy

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Introduction: Autophagy provides intracellular self-renewal and energy repletion through degradation of dysfunctional/misfolded protein. Ca^{2+}seems to play a critical role in autophagy regulation. In non-excitable cells, depletion of $[Ca^{2+}]_i$ stores triggers store-operated Ca^{2+} entry (SOCE). However, in neonatal cardiomyocytes this mechanism was not understood until the identification of STIM1 as the ER/SR Ca^{2+} sensor responsible for activation of Ca^{2+}release activating channel subunit (Orai1). The role of STIM1 on cardiomyocyte autophagy remains unknown and only a recent study shows that the STIM1 inhibition modulates prostate cancer cellautophagy. The aim of the present work is to evaluate the role of STIM1 and Orai1 in the regulation of cardiomyocyte autophagy. **Methodology:** Neonatal rat ventricular cardiomyocytes (NRVM) were cultured with DMEM/M199 media with 2% FBS or RPMI 1640 medium to study basal and starvation-induced autophagy, respectively. The levels of LC3II, GAPDH, STIM1 and Orai1 were assessed by Western blot.LC3 positive autophagosome were detected by confocal microscopy imaging using LC3GFP. **Results:** The results showed that LC3II levels increased significantly after 4 h of glucose deprivation assessed both Western blot and LC3-GFP by confocal microscopy. This effect was further confirmed by autophagy flux where LC3II level were accumulated after treatment with bafilomycin A1. Both STIM1 and Orai1 protein levels were significantly decreased upon autophagy induction. **Conclusions:** The results suggest that there is an inverse relationship between the STIM1-Orai1 pathway and cardiomyocyte autophagy.

142) Up-regulation of Akt/mTORC1 signaling by CK2 and its consequences in clonogenic potential of human colon cancer cells

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Introduction and objective: The Akt/mTORC1 pathway is one of the most commonly dysregulated pathways in cancer. Akt/mTORC1 has been recently proposed as a key factor in colon cancer by promoting viability and tumor growth. In this pathway, Akt activates by phosphorylation to mTORC1. Also, CK2 hyperactive Akt by phosphorylation at S129, however, whether increased mTORC1 activity in colon cancer cells is due to increased expression and activity of CK2 is unknown. Therefore, we studied the role of CK2 in promoting Akt/mTORC1 signaling and thereby *in vitro* proliferation and colony formation in human colon cancer cells. **Material and Methods:** We overexpressed full-length mTOR wild type (WT), Akt-WT and Akt-S129A (mutant unable for CK2-phosphorylation) in DLD-1 human colon cancer cells. CX-4945, triciribine and rapamycin were used to inhibit CK2, Akt and mTOR, respectively. Anchorage-independent colony formation was evaluated in soft agar and viability was measured by MTS® assay. Activation of mTORC1 was determined detecting the p-S6K1 and p-S6 proteins by western blot. Proliferation and apoptosis assays were evaluated by flow cytometry. **Results:** As expected, inhibition of either Akt-WT or mTOR decreased viability and colony formation. Interestingly, CK2 inhibition reduced mTORC1 activity as well as viability and colony formation. However, overexpression of mutant Akt-S129A did increase colon cancer cell viability in a similar way than Akt-WT. Finally, CK2 inhibition reduced cell viability promoted for overexpression of either Akt or mTOR in DLD-1 cells. **Conclusions:** CK2 may activate mTORC1 and promote clonogenic potential in human colon cancer cells. Therefore, this suggests that CK2 is a potential target for diagnosis, prognosis and treatment of this disease. **Keys Words:** CK2, Akt, mTORC1, cancer

143) Urinary exosome: Searching of novel potential markers for diabetic nephropathy

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Introduction: Exosomes are 40 to 100 nm-diameter vesicles released by cells and are formed within multivesicular bodies in the endosomal system. Normal human urine contains large numbers of exosomes, which are secreted by the epithelial cells of the urinary tract, and their isolation can result in marked enrichment of low abundance urinary proteins that have potential pathophysiologic significance. Previous studies from our laboratory have shown the kidney damage that in a model of streptozotocin-induced diabetic rat. Our current goal is to elucidate the putative protein markers involved in diabetic nephropathy by urinary exosomes isolation.

Methodology: Urine was collect from control and diabetic rats with/without kidney damage and exosome isolation was performed through of ultracentrifugation, and the vesicles morphology were analyzed by electronic microscopic. For clinical biomarker discovery, LC-MS based large-scale quantitative proteomic analysis was realized.

Results and conclusions: Diabetic nephropathy is the leading cause of kidney failure in Chile and the world. Therefore, it is necessary to identify new markers that are capable of detecting early stage for this disease. Our results show a significant difference in the protein content of healthy control rat-urine exosomes regarding early diabetic rat. In addition we could detect an increase in proteins isolated from urine exosomes diabetic rat of 8 months. These results suggest that urinary exosomes can be a useful tool in the identification of new markers associated with diabetic nephropathy (Innova-Corfo 13IDL2-23502)

144) Determination of the physical interaction and stoichiometry of 5-HT3$_{A/B}$ and P2X$_2$ receptors complex.

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Ionotropic 5-HT$_3$ and P2X$_2$ receptors mediate communication through rapid synaptic transmission in the central nervous system. Although it has been shown crosstalk between these two types of receptors, it is not known whether physical interaction and a defined stoichiometry can exist between 5-HT$_{3A/B}$ and P2X$_2$ receptors. HEK293 cells were transfected with plasmid DNA of 5-HT$_{3A}$, 5-HT$_{3B}$ and P2X$_2$ receptors tagged with Myc/His-6, V5/his-6 and GFP respectively. Membrane fractions were solubilized and incubated in Ni^{2+} agarose beads. Purified proteins were eluted with increasing concentrations of imidazole. 5-HT$_{3A/B}$-P2X$_2$ receptor complexes were analyzed by inmunoblotting, immunofluorescence and atomic force microscopy (AFM). Results suggest that both receptors are expressed in the plasma membrane and physical interaction in stoichiometric ratio can be present.

145) Increased tolerance to drought in *Arabidopsis thaliana* inoculated with *Burkholderia phytofirmans* PsJN: bacterial emission of volatile compounds and modulation of plant stress signaling

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Drought is a key environmental stress that limits the growth of plant crops worldwide. However, interaction with certain plant growth promoting rhizobacteria (PGPR) can improve plant growth in the presence of water stress. *Burkholderia phytofirmans* PsJN is a well-studied PGPR, reported to produce hormone phytostimulation of different plant hosts. We have shown that strain PsJN is able to enhance growth and drought tolerance of *Arabidopsis thaliana*, improving biomass yield in plants under low irrigation regimes. However, the bacterial functions involved in this effect are yet unclear. The aim of this work was to assess the role of specific signals and enzymes from *B. phytofirmans* on plant tolerance to drought stress, and to study the host physiological response underlying PGPR-induced stimulation. To accomplish this, Arabidopsis plants have been subjected to varying drought stress intensities. Rosette area, growth rate, and fresh/dry weight were measured, and gene expression of several plant stress response markers was assessed by qRT-PCR. We compared the growth and response to stress of *A. thaliana* inoculated directly with strain PsJN, and of plants exposed only to volatile emissions from the bacterium. Additionally, the contribution of bacterial ACC deaminase, was assessed using PsJN mutant derivative strains. The results of this work indicate that PsJN-inoculated plants become less sensitive to drought than their non-inoculated counterparts, as they display significantly lower expression of certain specific drought response genes. On the other hand, growth stimulation and tolerance under drought stress was found to depend on emission of volatile compounds rather than tissue colonization.

146) Rational design and Directed Evolution of 6-phosphogluconate dehydrogenase of *Escherichia coli*

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The 6-phosphogluconate dehydrogenase (6PGDH) from *Escherichia coli* catalyzes the decarboxilation of 6-phosphogluconate to produce ribulose 5-phosphate, using NADP as cofactor with high specificity. Previous studies attempted to switch the cofactor-specificity of 6PGDH to produce NADH under physiological conditions. Site-directed mutagenesis of residues in the β2-α2 loop of 6PGDH, which forms the NADP 2'-phosphate binding pocket, increased the NADP K_M without favoring the NAD affinity. In this study, rational design and directed evolution methodologies were implemented in order to obtain the NAD-specific form. Evolutionary trace analysis of the 6PGDH family was performed, finding 3 loops that could be relevant for NAD(P) specificity, including the β2-α2 loop containing the NRX_3K motif. From this analysis, the variant N33D-R34V-S35K-K38N was generated. Due the presence of these substitutions the NAD K_M decreased 5-times and no activity was observed with NADP as cofactor. Further increase of NAD affinity was attempted by generating a clone library by site-saturation mutagenesis of 4 residues within the 3 loops. In addition, a selection strategy was implemented using a Δ*pgi* Δ(*edd-eda*) Δ*udhA* Δ*gnd E. coli* strain that is unable to grow in minimal medium unless it is complemented with a NAD-producing 6PGDH. Clones of the library were compared with the strain bearing N33D-R34V-S35K-K38N 6PGDH by growth rate and enzymatic activity. Since growth rate is related to the NADH production, positive variants can be easily discriminated within the library. Further enzymatic characterization of NAD K_M of the selected clones will be performed.

147) Differential interaction of CCAAT/enhancer-binding protein beta (C/EBPβ) isoforms with the nuclear proteins XPC, HLTF and NF1C.

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The transcription factor C/EBPβ is involved in various cellular processes such as proliferation, differentiation and metabolic processes. It is found as three isoforms (C/EBPβ1, C/EBPβ2 and C/EBPβ3) generated as a consequence of translation at alternative initiation codons of a unique mRNA. The shortest isoform, C/EBPβ3, lacks the whole transactivation domain and acts mostly as a repressor. Conversely, C/EBPβ1 (the longest isoform) and C/EBPβ2 usually act as activators, but can also act as repressors depending on the cellular context. We have previously performed a proteomic screening, searching for new C/EBPβ interactors, using GST pull-down coupled to mass spectrometry analysis. By this mean, we found several candidate interactors, including HLTF (an ATP-dependent chromatin remodeling complex), XPC (a component of a DNA damage repair complex, recently found to participate in transcriptional regulation) and NF1C (a multifunctional transcription factor). Aiming to confirm these physical interactions, we carried out GST pull-down assays coupled to western blot detection, using affinity-purified recombinant C/EBPβ isoforms as bait and nuclear extracts obtained from HepG2 and HeLa cells or affinity-purified recombinant NF1C. HLTF and XPC were detected using specific antibodies. Our analyses show that the affinity of C/EBPβ long isoforms for XPC and NF1C is higher than that of C/EBPβ3, while a similar interaction strength of all C/EBPβ isoforms for HLTF was observed. We also performed pull-down assays in the presence of ethidium bromide to discard interactions through contaminant nucleic acids. Our results confirm the existence of new C/EBPβ interactions that might be linked to its roles on transcriptional regulation.

148) Metagenomic analysis of pico- and nano-planktonic communities from surface coastal waters from Bahia Fildes, King George Island, Antarctica.

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The study of the Antarctic ecosystem has become of great interest because of its importance for the regulation of atmospheric CO2 concentration, accounting for nearly 30% of global ocean uptake, while representing no more than 10% of the oceans total surface area. This CO2 uptake, along with other important key functions in the maintenance of the ecosystem, is mainly performed by microorganisms that inhabit the water column, transforming them in the focus of this research. The objective is to shed light on the microorganisms that are part of Antarctic surface coastal waters and understand the roles they may be fulfilling in this system. For this, we conducted metagenomics analysis, starting with Illumina sequencing of size-fractionated planktonic communities, followed by computational inspection of the data. The reads were separated into ribosomal DNA sequences and non-ribosomal sequences, using the first for taxonomic and the later for functional assignment. We found, in accordance with previous studies, an important abundance of Alphaproteobacteria and Gammaproteobacteria in the small fraction, and complex eukaryotic communities in the nano-planktonic fraction. Genes with functions predominantly associated with photosynthesis and carbon cycling were present in both size fractions. This investigation allows us to better understand the participation of the microorganisms present in surface coastal waters in the maintenance of chemical conditions and trophic interactions in the ecosystem they inhabit.

149) Immune response of the skeletal muscle in the fine flounder (*Paralichthys adspersus*)

Valenzuela, C[1]., Zuloaga, Rodrigo[1]., Cruz, Katherine[1]., Avendaño-Herrera, Ruben[2]., Poblete-Molares, Matias[2]., Irgang, Rute[2]., Valdes, Juan[1]., Molina, Alfredo[1]., [1]Laboratorio de Biotecnologia Molecular Universidad Andrés Bello. [2]bLaboratorio de Patología de Organismos Acuáticos y Biotecnología Acuícola Universidad Andrés Bello. (Sponsored by Supported By FONDAP 15110027 And FONDECYT 1130545)

Introduction: The key immune molecules involved in the detection of foreign agents are the pattern recognition receptors (PRRs), which recognize a variety of pathogens associated molecular patterns (PAMPs) such as lipopolysaccharides, peptidoglycan and viral nucleotides, among others. Among PRRs, we found toll-like receptors (TLR), leucine-rich repeat containing receptors (NLRs) and the retinoic acid inducible gene I (RIG-I) like receptors (RLRs). In fish, several TLR, NLR and RLR have been characterized. However, the studies have addressed only in organs that are associated to the immune response, including kidney, spleen, liver and/or gills. The ability to trigger an immune response of the skeletal muscle, which represents more than 50% of the body, has not been studied in detail until now.

Methodology: The aim was to assess the PRRs expression under *Vibrio ordalii* challenge to make a descriptive analysis of PAMPs detection machinery in the fine flounder skeletal muscle. Fish (6–10g) were randomly allocated in 7 groups of 20 per 50L tank and one-week acclimatized to bacterial challenge. 3 groups were inoculated by intraperitoneal injection with 0.1ml of high, medium and low dose of infection ($2.59 \times 10^{7, 5, 3}$CFU/fish, respectively) using the Vo-LM-18 strain. Another group were bath separately in seawater with 2.59×10^{7}CFU/ml. Control fish received 0.1 ml of TSB or the same amount of broth was added, while another group of fish was not manipulated. To determine the PRRs gene expression, fine flounder were removed from each tank after 2, 4 and 10 days post-infection and skeletal muscle samples analyzed by qPCR.

Results and Conclusions: The immune response triggered by *V. ordalii* challenge, has a positive effect on the expression of the receptors responsible for pathogen recognition in skeletal muscle of fine flounder, showing that the muscle is an active organ when facing pathogens.

150) Analysis of the mechanisms by which the yeast HMG protein Nhp6 favors the association of the ySWI/SNF complex to gene regulatory regions

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Nucleosomes generally restrict the access of DNA-binding proteins to their cognate sites. Chromatin modifying machines facilitate the access of DNA-binding proteins to the DNA in this chromatinic context. Among these machines are ATP-dependent chromatin remodeling complexes, including SWI/SNF (switching defective/sucrose non-fermenting). This complex is involved in transcriptional regulation of several genes in yeast. High Movility Group Box (HMGB) proteins also play a significant role in chromatin dynamics. In this context, using *in vitro* nucleosome remodeling assays we have previously demonstrated that the yeast HMGB proteins Nhp6 and Hmo1 stimulate ySWI/SNF activity. However, only Hmo1 can stimulate the association of this complex to the nucleosome. Further *in vivo* studies, analyzing the genome-wide association of ySWI/SNF to gene promoters in yeast, showed that Hmo1 is required for the association of ySWI/SNF to regulatory regions of several genes. Surprisingly, Nhp6 was also required for association of this complex to a large number of genes. We reasoned that Nhp6 could influence the transcription factor-mediated association of ySWI/SNF to gene promoters. Considering this possibility, we performed EMSA assays analyzing the influence of Nhp6 on Gal4-VP16-mediated association of ySWI/SNF to a mononucleosome. These analyses showed that Nhp6 can enhance targeting of SWI/SNF to the nucleosome by this chimeric transcription factor. In addition, we validated our previous genome-wide studies by performing ChIP-qPCR assays and RT-qPCR assays for selected genes. Taken together, our results indicate that Nhp6 contribute to the association of ySWI/SNF to gene regulatory regions by assisting the recruitment of this complex by transcription factors.

151) Biophysical characterization and preliminary crystallization assays of the forkhead domain of the transcription factor Fhl1, a key regulator of yeast ribosomal protein genes.

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Forkhead box proteins (Fox) constitute a family of transcription factors that are fundamental for gene regulation and cell homeostasis in eukaryotes. Mutations on the DNA-binding domain of human Fox proteins are related to several inherited diseases. In yeast, four Fox proteins (Fkh1, Fkh2, Hcm1 and Fhl1) participate in chromatin silencing, cell cycle regulation, stress response and replication origin timing. Particularly, Fhl1 binds almost exclusively to promoters of ribosomal proteins and is fundamental for their synthesis. During the last decade, several structures of DNA-binding domains of human Fox proteins have been solved, with some of them corresponding to monomers and others to domain-swapped dimers (DS), where the C-terminal region is exchanged with an adjacent subunit. This is allowed by a Pro-to-Ala substitution at the hinge region connecting the exchanged elements. DS has been suggested as a compelling mechanism in yeast to bridge two DNA replication origins and mediate interactions in trans, but a biophysical characterization of yeast Fox proteins remains to be elucidated. Here, we biophysically characterized for the first time the DNA-binding domain of Fhl1. Its aggregation state ascertained by size exclusion chromatography corresponds to a monomer in solution, as expected due to the presence of a proline residue in the hinge region. Then, we performed equilibrium-unfolding experiments, determining that Fhl1 follows a two-state folding mechanism with a stability of 6.4 kcal/mol, similar to other Fox proteins. Finally, we performed a high-throughput exploration of crystallization conditions, obtaining crystals in 2-methyl-2,4-pentanediol or 30% v/v 2-propanol and magnesium salts. FONDECYT 11140601 & 1130510

152) Characterization of DYRKs kinases in seminiferous epithelium and its possible role as activity regulator of MGS.

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Dual-specificity tyrosine-phosphorylation-regulated kinases (DYRKs) belong to the CMGC family of serine/threonine kinases. They are categorized as class I (DYRK1A and DYRK1B) and class II (DYRK2, DYRK3, and DYRK4) and have roles in regulating transcription, cell-cycle progression, differentiation and cell survival. DYRKs are distributed in many tissues, including testis but there is no information about the cellular distribution and functions in this organ. DYRK cellular substrates have a wide variation in phosphorylation motifs, in the case of muscle glycogen synthase (MGS), DYRK1A and DYRK2 phosphorylate serine641 which leads to the inactivation of this enzyme. The aim of this work was to characterize DYRK family in two cellular components of seminiferous epithelium, specifically Sertoli and spermatogonia cell-type and study its potential regulatory role on MGS. By qRT-PCR we determined differential expression of DYRKs, being DYRK1A the main kinase in Sertoli and germ cell in comparison with testis, where DYRK1B is highly expressed. Western Blot analysis revealed that all DYRKs analyzed are present and we detected differences independently of the transcript level. Immunofluorescence analysis exhibited a particular cytoplasmic pattern of DYRK1A in both cell types distinct to DYRK1B, which was homogenously distributed in the cytoplasm. *In vitro* kinase assay revealed that in Sertoli and germ cells DYRK1A is active. Finally, co-immunoprecipitation showed that DYRK1A and DYRK1B interact with glycogen synthase. These findings suggest that in seminiferous epithelium, variations of DYRKs could have various functions, in addition to MGS regulation.

153) Characterization of gamma^{31} subunit associated to phycoerythrin from *Gracilaria chilensis*

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Linkers proteins are associated to phycobiliproteins in phycobilisomes. One of the linker protein associated to phycoerythrin is chromophorilated and it has been classified as a γ^{31} subunit. There are evidences suggesting its role in the assembly of the complex and in the fine tuning of the energy transfer.

The gene encoding sequence for linker protein γ^{31} was obtainedby 5 ‘and 3’ Rapid Amplification of cDNA Ends (RACE). The *in silico* translation generated the primary structure consisting of 280 amino acid residues. Their secondary structure was predicted by PSIPRED server and models by I-TASSER and ROBETTA servers were generated. The sequence was confirmed by mass spectrometry (MALDI-TOF) and the protein was characterized by fluorescence spectroscopy. The sequence analysis detected a chloroplast transit peptide signal at the N-terminus and a repetition of the M67 - T170 regions with the S179 -F273 region.The secondary structure prediction showed only helical structures, as well as the models showed similar folding as α-solenoid motif. The γ^{31}sequence showed 54%, 50% and 48% of identity with γ subunits of *A. neglectum*, *Chondruscrispus* and *G. japonica*, respectively and four conserved cysteines residues as potential sites for chromophorilation. The spectroscopic analysis suggests the presence of phycoerythrobilin (PEB) and phycourobilin (PUB) attached to the protein.

154) Structural analysis and SDS-induced phenol oxidase activity of hemocyanins from the Chilean Theraphosidae spiders *Grammostola rosea* and *Euathlus condorito*.

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Hemocyanins are hetero-multimeric giant proteins of the hemolymph of many invertebrates, like arthropods and molluscs that play a central role in oxygen transport. These proteins bind the O_2 molecule in a highly conserved type 3 copper center, which can also be found in tyrosinases and catechol oxidases. Interestingly, hemocyanins exhibit phenoloxidase (PO) activity *in vitro*, after activation with micellar concentrations of sodium dodecyl sulfate (SDS). *In vivo*, PO activity is necessary for melanin synthesis, sclerotization and immune innate response. However, specific tyrosinases or cathecol oxidases have not been found up to date in Chelicerata, suggesting that hemocyanins could be fulfilling this role *in vivo*. Indeed, PO activity was demonstrated for several arthropod hemocyanins. In the present work, the hemocyanins from two Chilean endemic spider, *Grammostola rosea* and *Euathlus condorito*, were characterized by enzyme kinetics and peptide fragment sequencing. Hemocyanin was purified from the respective hemolymph in a single step of molecular exclusion chromatography. Aminoacid sequence analysis obtained by MALDI-TOF MS and LC-ESI-MS/MS, showed partial coverage for different subunits, matching the sequence of the hemocyanin from the Theraphosidae spider *Aphonopelma californicum*. PO activity was determined spectrophotometrically measuring the production of dopachrome, the dopamine oxidation product. Hemocyanins from both species showed catechol oxidase activity only after SDS activation, at least 20-fold enhancement in the presence of divalent cations, as Ca^{+2} or Mg^{+2} and substrate inhibition above 4mM dopamine. Comparative analysis of these results suggests conservation of structure and function among Chelicerata hemocyanins.

155) IMPAIRED INSULIN RESPONSE IN UMBILICAL CORDS FROM MATERNAL OBESITY PREGNANCIES

Villalobos-Labra, Roberto[1,2]., Westermeier, Francisco[1,2,3]., Sáez, Pablo[1,2]., Pizarro, Carolina[1,2]., Kusanovic, Juan[1]., Poblete, José[1]., Mardones, Francisco[4]., Sobrevia, Luis[1,2]., Farías-Jofré, Marcelo[1,2]., [1]Obstetrics and Gynaecology, School of Medicine, Medicine, Pontificia Universidad Católica De Chile. [2]Cellular and Molecular Physiology Laboratory (CMPL), Medical Research Centre (CIM), School of Medicine, 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, Medicine, Pontificia Universidad Católica De Chile. (Sponsored by FONDECYT (1121145, 1110977, 1150377, 1150344)) Maternal obesity (MO) has been recognized as a risk factor for maternal and fetal complications, including offspring´s insulin resistance (IR) later in life. Until now, little has been studied regarding IR in newborn. Herein we evaluated the effect of MO in endothelial cells and umbilical vein vasodilatation in response to insulin. Primary cultures of human umbilical vein endothelial cells (HUVEC) and rings were isolated from normal (HUVEC-N) or MO (HUVEC-OB) pregnancies attending to obstetrics clinical service at Pontificia Universidad Católica de Chile Hospital. We evaluated the basal phosphorylation and total protein levels of eNOS by western blot analysis. The NO production by HUVEC and vasodilation of umbilical vein rings in response to insulin was evaluated by fluorescence and wire myography, respectively. We found that total eNOS and the activating phosphorylation on Ser1177 was reduced in HUVEC-OB compared to HUVEC-N. Conversely, the inhibitory phosphorylation on Thr495 was increased in HUVEC-OB. Also, HUVEC-N exposed to insulin (1nM) showed increased levels of NO at 5, 15 and 30 min of incubation, an effect blocked by the inhibitor of NOS L-NAME. In contrast, insulin did not increase NO production in HUVEC-OB. Finally, vein rings from MO showed less relaxation in response to insulin than rings from Normal pregnancies, effect blocked by L-NAME incubation. In this study we have shown evidence that MO promotes less vasodilation of umbilical vein in response to insulin partly due to the higher inhibitory state of eNOS and the consequent lack of NO production by HUVEC.

156) Genome-wide chimeric-transcript discovery in Wnt/β-catenin stimulated human hematopoietic precursor cells.

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Introduction: Chromosomal translocations are frequently associated with a wide variety of cancers, particularly hematologic malignancies. Recent evidence indicates that β-catenin promotes genomic instability and cell transformation in mice by compromising DNA repair and enhancing illegitimate recombination. Here we used high-throughput sequencing to search for chimeric transcripts in hematopoietic precursors under sustained Wnt signaling stimulation. Materials and Methods: Total RNA was extracted from human CD34+ hematopoietic cells incubated with or without purified Wnt3a protein (200 ng/mL; 48 h). RNA (RIN value > 8) was reverse-transcribed into cDNA and processed using the Illumina TruSeq Total Stranded RNA-seq with Ribo-Zero ribosomal RNA depletion kit. Libraries were sequenced using the Illumina Hi-Seq2500 with a depth > 120 million pair-end reads (2x50 bp) following ENCODE guidelines. The resulting data was analyzed with FusionCatcher (v.0.99.c) using default, highly sensitive and “paranoid” analysis parameters. Results: We found a consistent increase in the number of chimeric transcripts in Wnt3a-treated CD34+ precursors compared with control cells. Among chimeric transcripts exclusive to Wnt3a-treated cells we detected 3 novel inter- and intra-chromosomal transcripts: MPO-CALR, MPO-SPN, RN7SL1-RN7SL40P; and 7 gene fusions: ASH1L-YY1AP1, BAHD1-IVD, ELMO1-AOAH, SQRDL-BLOC1S6, RCC1-UBE2D2, UCHL3-LMO7, USP34-XPO1 (which are already known in TCGA database or the literature). Interestingly, MPO-positive blast cells are a strong prognostic factor in acute myeloid leukemia. Discussion: Our results indicate that Wnt/β-catenin signaling enhances gene-fusion events in hematopoietic cells supporting a link between sustained activity of the cascade and the onset/development of leukemia. Funding: Anillo ACT-1119, FONDECYT 3130509 and 1140353, IMII P09-016-F, FONDAP 1509000.

157) CK2 regulates autophagy via activation of the Akt/mTORC1 pathway in colon cancer cells

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Introduction and objective: CK2 is a highly conserved Ser/Thr kinase whose deregulation has been linked to the progression of numerous cancers. CK2 is also involved in different signaling pathways, including PI3k-Akt where hyperactives Akt by phosphorylation at Ser129. A target of Akt is mTORC1, a complex that integrates multiple signals and processes, including inhibition of autophagy, which has been associated with cancer progression. Pharmacological inhibition of CK2 with CX-4945 induces autophagy in glioblastoma and lung cancer cells, however, CK2/Akt-dependent autophagy in colon cancer cells has not been studied yet. **Material and Methods**: DLD-1 human colon cancer cells were used as a model. CK2 was inhibited by CX-4945. Autophagy was assessed by detecting augmented LC3-II protein levels by western blot. Confocal microscopy was used to visualize GFP-LC3 localization. CK2a subunit, Akt and Akt-S129A (a mutant non-phosphorylatable by CK2) were overexpressed to evaluate their effects in autophagy. **Results:** CX-4945 induced autophagy as observed by increased LC3-II protein levels. Likewise, GFP-LC3 fluorescence changed from cytosolic to punctuated distribution in presence of CX-4945, which suggested autophagosome formation and thereby autophagy induction. Overexpression of CK2a and Akt in presence of CX-4945 lead to partial augmented LC3-II levels compared with the untreated control. Interestingly, Akt-S129A in the presence of CX-4945 has no significant change in levels of LC3-II compared to untreated control. **Conclusion:** CK2 inhibition promotes autophagy in human colon cancer cells, presumably due to the activation of the Akt/mTORC1 pathway.

158) MITOCHONDRIA COULD BE THE INTRACELLULAR TARGET OF GLUCOSE POLYMERS-INDUCED APOPTOSIS IN MALE GERM CELLS

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In most tissues, glucose is stored as large intracellular polymers (or polyglucosan) to support several metabolic processes; however, its accumulation can lead to degeneration and death in some cell types. Previously, we reported that the accumulation of polyglucosan polymers enhances the apoptotic rate in testis. In this report, we show the effects of glucose polymers in spermatocyte-like cells (line GC-1) and the mechanism involved in the pro-apoptotic activity induced by their accumulation. Under conditions of glycogen accumulation in GC1 cells we immunodetected the active form of caspase -3 and -9 and an increase in the levels of free cytochrome C, suggesting that polyglucosan molecules could promote mitochondrial impairment and activation of an intrinsic apoptotic pathway. Isolated mitochondria were incubated in presence of amylopectin or glycogen, and the swelling effect induced by these polymers was determined by spectrophometric analysis. High levels of glucose polymer affect directly the mitochondrial morphology and show a significant increase of absorbance. Calcium released from mitochondrial reserve was measured as a change in calcium-green fluorescence. The results show higher levels of free Ca2+ as a function of the amount of amylopectin and glycogen used in each assay. Glucose and sucrose were used as controls for osmolar and poly-hydroxide group effects in the experiments with isolated mitochondria. These results suggest that glucose polymers can promote impairment on the morphology and permeability of mitochondria, and that the accumulation of polyglucosan molecules could have a selective effect triggered by the intrinsic activation of the apoptotic pathway in male germ cells.

158) Anticancer activity of a ruthenium complex in gallbladder carcinoma cells

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Introduction: Gallbladder cancer (GBC) is highly prevalent in Chile, with higher incidence than the rest of world. Moreover, the survival rate is low and it prognosis is poor in advanced stages of carcinoma. At this respect, the search for new drugs with better activity and lower side effects is necessary. Ruthenium compounds represent an alternative for the development of more efficient drugs for the treatment of GBC. The objective of this study was to determine the anticancer activity of a new complex based on ruthenium(II), on the GBC cell line, G-415.

Methods: G-415 cells were grown on 96 well plates and different concentrations of ruthenium were added, from 1.0 to 100mM. Cellular viability was assayed by the MTT method. The expression of genes p53, Bax and caspase-3 was evaluated by qPCR in cells treated with 25 and 75mM of Ru(II).

Results: The MTT assay revealed a significant decrease in the cell viability that is dose dependent with an IC50 of 20.97uM. The gene expression analysis showed a significant increase in the expression of caspase-3 with both treatments, meanwhile the expression of p53 only increased with 25 mM of Ru(II). The expression of Bax was lower in both treatments respect to the untreated control.

Conclusions: The results indicate that the ruthenium(II) complex has evident antiproliferative activity and induces an increasing expression of pro-apoptotic genes in the cell line G-415. Our results indicates that the ruthenium complex synthesized in our laboratory can be an potential agent for the treatment of GBC.

160) Adenosine receptor 1 mediates insulin-induced glucose uptake in adult rat cardiomyocytes

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Insulin has pleotropic actions in the heart by controlling contractility, glucose and lipid metabolism, cardiomyocyte hypertrophy and death. Conversely, its deficiency is associated with impaired myocardial function. The cardioprotective role of adenosine has been described, however, there is no evidence regarding a potential functional link between adenosine and insulin signaling in the heart. We evaluate here the expression of adenosine receptor sub-types A_1, A_{2A}, A_{2B}, and A_3 by quantitative PCR, Western blot and immunofluorescence studies in isolated adult rat cardiomyocytes and also by immunohistochemistry in paraformaldehyde-fixed hearts. Our results showed that $A_{2A}>A_1$ exhibited a predominant expression compared to $A_3>A_{2B}$. Interestingly, the A_1 antagonist N6-Cyclopentyladenosine (CPA) blocked the insulin-stimulated glucose uptake in isolated adult rat cardiomyocytes. We conclude that adenosine trough activation of A_1 AR mediates insulin-induced glucose uptake in adult rat cardiomyocytes, suggesting a novel role of adenosine in cardioprotection.

161) Isolation and characterization of Outer membrane vesicles (OMVs) produced by *Piscirickettsia salmonis*.

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Introduction

Piscirickettsia salmonis is a Gram-negative bacterium that causes Piscirickettsiosis in all farmed salmonids in Chile. Recently, it has been reported that *P. salmonis* produce exotoxins that play a role in the bacterial pathogenesis. OMVs are 10-300 nm spherical-bilayer structures discharged from the surface of many Gram-negative bacteria, which are able to deliver virulence factors into the host cell. Thus, the aim of this study was to investigate if *P. salmonis* is able to produce OMVs and to evaluate its role in pathogenesis *in vitro.*

Methodology

OMVs isolation: *P. salmonis* LF-89 (ATCC VR-1361) was grown in 400 mL of a minimal broth at 18°C until early stationary phase. Bacteria were removed by centrifugation and the resultant supernatant was filtered through a 0.2-mm/pore filter. Finally, OMVs were isolated by ultracentrifugation (125,000 x g, 2h at 4°C) and stored at -80°C until use. Electron microscopy: infected cells and isolated OMVs were fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer for 2h at RT and postfixed with 1% osmium tetroxide in 0.1 M CB. All samples were visualized with a JEOL JEM-1320 transmission electron microscope. Cytotoxicity assay: CHSE-214 cells were incubated in 1 ml of OMVs-containing medium for 4 days at 18°C.

Results and conclusions

P. salmonis produces OMVs during infection in CHSE-214 cells and during normal growth in liquid media. Protein analysis demonstrates the pattern of OMVs were similar to outer membrane protein profile. Finally, infection assays indicate that purified OMVs induce cytotoxic effect in a salmonids cell line.FONDAP-INCAR 15110027.

162) Evolution of structural patterns on ADP-dependent extremophile enzymes: Biophysical and evolutionary study of their structural flexibility.

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In extremophile enzymes,several sequence and structure features are associated with a propensity of higher structural flexibility. The structural flexibility is a property that is common in cold-adapted enzymes, which perform their metabolic processes at low temperatures (psychrophilic enzymes). During the evolution, the general mechanism adopted by these enzymes is to perform catalysis at low temperature by reducing the free energy of the transition state ($DG^{\ddagger}$), rather than the Michaelis constant K_m. A correlation between increased structural flexibility and decreased affinity for its substrates has been shown in psychrophilic enzymes, a phenomenon that is compensated by an increase of the catalytic rate (k_{cat}) at low temperatures. At relevant physiological temperatures, the K_m values reported for the natural substrates of psychrophilic enzymes are generally greater than those obtained for their thermostable counterparts, although there are a few examples of psychrophilic enzymes having lower values of K_m, than thermostable enzymes. In the ADP-dependent kinase sugar family, we have recently identified a psychrophilic enzyme from *M. burtonii* that has particular structure and sequence features that make this enzyme more flexible than their thermostable homologues. In this study, we characterize the bifunctional psychrophilic enzyme phosphofructokinase/glucokinase from *Methanococcoides burtonii* (*Mb*PFK-GK) and the bifunctional mesophilic enzyme phosphofructokinase/glucokinase from *Methanococcus maripaludis* (*Mm*PFK-GK) by spectroscopic (circular dichroism), biophysical (hydrogen-deuterium exchange coupled to mass spectrometry) and computational (phylogenetic analysis and classical molecular dynamics) techniques. The comparison between psychrophilic and mesophilic enzymes showed that the presence of two ion pairs are primarily responsible for the increased structural flexibility accounted in the psychrophilic model. Additionally, through bayesian inference we reconstruct the sequences of all ancestral enzymes between the current enzymes and their last common ancestor, which was used to trace the occurrence of these electrostatic interactions in all the enzymes along the phylogenetic tree of the ADP-dependent sugar kinase family. Our results suggest that the electrostatic interactions in this protein family are a dominant feature of transition to psychrophilic or thermophilic environments.

163) Evolution of structural patterns on ADP-dependent extremophile enzymes: biophysical and evolutionary study of their structural flexibility.

Zamora, R[1]., Castro-Fernandez, Victor[1]., Ramírez-Sarmiento , Cesar[1]., Komives, Elizabeth[2]., Guixe, Victoria[1]., [1]Departamento de Biologia, Facultad de Ciencias, Universidad De Chile. [2]Department of Chemistry and Biochemistry University of California San Diego. (Sponsored by Fondecyt 1150460)

In extremophile enzymes several sequence and structure features are associated with a propensity of higher structural flexibility, a common property of cold-adapted enzymes, (psychrophilic enzymes). During the evolution, the general mechanism adopted by these enzymes is to perform catalysis at low temperature by reducing the free energy of the transition state ($\Delta G^{\ddagger}$), rather than the Michaelis constant. Increased structural flexibility and decreased affinity for its substrates compensated by an increase in the catalytic rate has been observed in psychrophilic enzymes. In the ADP-dependent kinase sugar family, we have recently identified a psychrophilic enzyme from *M. burtonii* that has features that make this enzyme more flexible than their thermostable homologues. In this study, we characterize the bifunctional psychrophilic enzyme phosphofructokinase/glucokinase from *Methanococcoides burtonii* (*Mb*PFK-GK) and the bifunctional mesophilic enzyme phosphofructokinase/glucokinase from *Methanococcus maripaludis* (*Mm*PFK-GK) by spectroscopic (circular dichroism), biophysical (hydrogen-deuterium exchange coupled to mass spectrometry) and computational (phylogenetic analysis and classical molecular dynamics) techniques. The comparison between psychrophilic and mesophilic enzymes showed that the presence of two ion pairs are primarily responsible for the increased structural flexibility accounted in the psychrophilic model. Additionally, through bayesian inference we reconstruct the sequences of all ancestral enzymes between the current enzymes and their last common ancestor, which was used to trace the occurrence of these electrostatic interactions in all the enzymes along the phylogenetic tree of the ADP-dependent sugar kinase family. Our results suggest that the electrostatic interactions in this protein family are a dominant feature of transition to psychrophilic or thermophilic environments.

164) microRNA expression profiling in breast cancer tumors associated to BRCA1 expression.

Zavala, Valentina[1]., Gajardo, Patricia[1]., Alvarez, Carolina[1]., Fernandez, Wanda[2]., Cornejo, Valeria[2]., Gamboa, Jorge[3]., Carvallo, Pilar[1]., [1]Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile. [2]Unidad de Anatomía Patológica Hospital San Borja Arriarán.[3]Unidad de Patología Mamaria Hospital San Borja Arriarán. (Sponsored by FONDECYT 1120200, CONICYT 21120269.

BRCA1 is a tumor suppressor gene which mutations confer high susceptibility to develop breast cancer. In addition, it has been described that 50% of hereditary breast cancer tumors with no germline mutation loss BRCA1 expression, as well as 30% of sporadic breast tumors. In this relation, different somatic events have been described that silence BRCA1 such as promoter hypermethylation and genomic deletions of BRCA1 locus. These mechanisms do not completely explain BRCA1 loss in breast tumors. miRNAs constitute a relevant mechanism in silencing gene expression, since they have been found deregulated in different cancer types. Our aim was to identify miRNAs differentially expressed in tumors with low/absent BRCA1 protein. We isolated total RNA from 36 fresh frozen breast cancer tumors, not selected for family history. miRNA profiling was performed using 8x15K Human miRNA Microarrays (Agilent Technologies). Raw data was background corrected, normalized using quantiles and log2 converted. We considered for analyses only probes with an intensity 10% over the brightness of negative controls. As reference, for expression levels we used a commercial RNA pool derived from normal mammary glands from 5 donors. Differentially expressed miRNAs among BRCA1 positive and BRCA1 negative tumors were detected using RankProd Package in R. Eighteen miRNAs were found upregulated in BRCA1-deficient tumors ($p<0.05$). Prediction analyses using mirWalk revealed eight miRNAs that could regulate BRCA1 expression. In addition, pathway analysis using mirPath showed that one overexpressed microRNA, miR-575, is implicated in the regulation of different cancer pathways such as MAPK and PI3K-AKT signaling and cell cycle pathways. Among the targets described for miR-575 we found: BRAF, E2F2, PIK3CB, FGF1 and MDM2 genes. Interestingly, it has been described that inhibition of PI3K-AKT pathway, through the silencing of PIK3CA or PIK3CB impairs BRCA1 expression and sensitizes breast cancer cell lines to PARP inhibitors. Our results suggest that miR-575 produce an indirect silencing of BRCA1, through the regulation of PIK3CB. In relation to the other miRNAs, mirPath analysis identified different targets associated to secondary metabolic pathways.

165) Contrasting oceanographic conditions regulate the expression of muscle atrophy and hypertrophy related genes in intertidal fish *Girella laevifrons*

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Introduction: The South-Eastern Pacific marine environment has contrasting physico-chemical conditions such as upwelling areas (U) (cold waters, high contents of nutrients), affecting marine habitat which have evolved adapting to this environment. Skeletal muscle is highly influenced by nutrition and abiotic parameters and the main molecule controlling its growth is IGF1, balancing between protein degradation (atrophy) and synthesis (hypertrophy). The aim was to assess intraspecific variations of genes related with muscle atrophy (*murf1, atrogin1, bnip3*) and hypertrophy (*igf1, myhc*) in *Girella laevifrons* from U and without upwelling (NU). **Methods:** Two trials, 1) six fish from U and NU were feed with 4 different diets: protein-rich, fiber-rich, high and low-mixture (A, B, C and D), and 2) six fish from both areas were fasted for 12 day and refed for 12 days. **Results:** 1) muscle *igf1* increase with high protein-fiber diet. *murf1* has a lower expression in U than NU, *atrogin1* upregulate in no mixture diets and *bnip3* only with fiber in NU. *myhc* decrease with fiber diet compare to high mixture in NU. 2) *igf1* decrease during fasting and increase during refeeding, same as *myhc*, but showing a higher upregulation at the end of this period in U. *atrogin1*, *murf1* and *bnip3* show an opposite effects with a overexpression in NU than U. **Conclusions:** *Girella laevifrons* has evolved in different oceanographic condition generating molecular rearrangements for muscle growth. This could reflect an evolutionary mechanism to adapt inhabiting in different environments, leading to muscle plasticity.

166) Construction and characterization of biological circuits to create a synthetic bacterial consortium

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Bacterial quorum sensing (QS) signal synthases, receptors, and cognate promoter elements are important components of a wide variety of engineered biological devices. We employed QS components to construct a bidirectional cell-to-cell communication network using three compartmentalized circuits to create a synthetic bacterial consortium. This strategy allowed us to obtain a better control of gene expression and has the potential to become a powerful biotechnology for various applications. We constructed a synthetic bacterial consortium, in three different *Cupriavidus pinatubonensis* JMP134 bacterial cell, creating a genetic variant monospecies consortium. *luxI* gene present in circuits of bacteria A and C and *rhlI* gene present in circuit of bacterium B, catalyze the synthesis of the acyl homoserine lactone (HSL), 3-oxododecanoyl-HSL and butanoil-HSL, respectively. When HSLs signal accumulate at high enough concentrations these molecules diffuse through the cell membrane and binds to their cognate transcription factor (LuxR in A and C and RhlR in B) and activate respectively the promoter. We characterized the promoter activity of each circuit in presence or not of each HSL by flow cytometry. The results suggest that genetic variants strains of *C. pinatubonensis*, expressing the constructed circuits, seem to have a predictable behavior. The data obtained can be used to model and simulate the consortium dynamics and predict the complex behaviors of bacteria in response to specific environments.

167) BraiNEXchange, Nexos Chile-USA internship program that promotes the collaboration between U.S. laboratories and Chilean Scientists

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Nexos Chile-USA has launched the internship program braiNEXchange, which aims to provide scientific opportunities for Chilean professionals in prestigious laboratories in the U.S. Offering positions for pre-doctoral stays and postdoctoral openings, braiNEXchange serves as the connection between prospective applicants and the principal investigators interested in working with motivated Chilean scientists. Applications will be open from October 2015 to March of 2016 for its second call. For more information, please visit the link http://www.nexoschileusa.org/#!brainexchange/c1hh

168) Identifying new transient receptor potential (TRP) channels in organisms from across the eukaryotic domain

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Transient receptor potential (TRP) channels are a subgroup of the voltage-gated ion channel superfamily. From insects to mammals, TRP channels play roles in cell signaling and in a wide spectrum of somatosensory modalities. To date TRP channels are grouped into seven families with low sequence identity among them. We recently discovered a new family of TRP channels in algae and present here a timely evolutionary update of the TRP subgroup that defines many novel TRP families.

First, we performed a search for TRP channels throughout the eukaryotic domain including genomes generated by consortiums focused on plants, birds and unicellular organisms. Starting from 68 *bona fide* TRP channels we identified sequences that shared global similarity, as well as local similarity with their transmembrane regions. Then, using sequence similarity networks (SSNs), we performed all against all comparisons of the sequences identified. Our work identified over 8000 uncharacterized members of the TRP subgroup in organisms from several eukaryotic phylum, among them, several members from algae. Using node centrality we selected representative sequences from clusters in the SSN to built a representative phylogenetic tree. We also studied conservation of domains across all TRP channels.

Our work reveals the evolutionary history of the TRP subgroup for over 1.5 million years, and charts a very vast sequence space of uncharacterized TRP families. Our results also reveal that TRP channels have a high level of conservation in their structural domains, and that novel families correspond mostly to recombinations of the domain present in the ancestral TRP channels.

169) Impact of benzo-a-pyren (BaP) on expression of pituitary factors and Cyp1A in hypophysis and liver of Cyprinus carpio

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Environmental contaminants such as BaP excert endocrine disrupting effects, however, the effect on pituitary factors is not well understood. BaP is a dioxin like molecule activating *cyp*1A expression via the arylhydrogen recepto pathway. Cytochrome P450 (CYP) belongs to a superfamily of mono-oxygenases, important for steroid hormon synthesis and also for detoxification by catalyzing transformation of polycylic-aromatic-hydrocarbons to excretable, harmless metabolites. With the aim to analyze the effect of BaP in vivo in a whole sentinel organism, we assessed gene expression in pituitary and liver of *Cyprinus carpio*. Fish were captured in the surroundings of Valdivia and maintained in a pond with flow through spring water at natural temperature and photoperiod. Adult male carp were injected intraperitoneally with 1mg/kg BaP (n=4), and controls with vehicle (oil/ethanol 9:1) (n=4) for 3 days and sacrificed on he ourth day. Total RNA was prepared and specific transcripts were quantified by RT-qPCR normalized for β-actin expression. Beforehand all amplicons were cloned and specificity was confirmed by sequencing. Clearly, *cyp*1A transcripts increased significantly in BaP treated respect to control carp, in liver as well as in pituitary. Differential effects were found for a series of hypophyseal factors including prolactin, growth hormone, pituitary transcription factor Pit1. Unexpectedly, a significant increase of somatolactin *sl*β, but not *slα* was determined suggesting differential regulation of these duplicated genes in response to BaP treatment. In comparison, no significant effect on Cyp1A enzymatic activity in liver was detected by ethoxyresorufin-deethylase-assay (EROD). Therefore, complementary assays revealed biologically relevant BaP impact in carp.

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