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XXXIX Reunión Anual de la Sociedad de Bioquímica y Biología Molecular de Chile

September 27-30, 2016

Puerto Varas, Chile

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LECTURES

Opening Lecture

From structure to function: the convergence of structure based models and co-evolutionary information

Onuchic, J. N[1]., [1]Center for Theoretical Biological Physics and Departments of Physics and Astronomy, Chemistry and Biosciences, Rice University.

Dr. José Onuchic is the Harry C & Olga K Wiess Chair of Physics and Professor of Physics and Astronomy, Chemistry and Biosciences at Rice University. He is also the co-Director of the NSF-sponsored Center for Theoretical Biological Physics (National Science Foundation of the USA) and a CPRIT Scholar in Cancer Research (Cancer Prevention and Research Institute of Texas). Dr. Onuchic's research looks at theoretical and computational methods for molecular biophysics, chemical reactions in condensed matter, and gene networks. He introduced the concept of protein-folding funnels to show the types of amino acid sequences that can fold into a unique protein structure. Dr. Onuchic and his collaborators also created the concept of tunneling pathways and the methodology for reducing proteins into a combination of relevant tubes of pathways that provides a new way of designing electron transfer proteins. He also focuses on stochastic effects in genetic networks in particular for bacteria and cancer. In 1989 he was awarded the International Centre for Theoretical Physics Prize in honor of Werner Heisenberg in Trieste, Italy, and in 1992 he received the Beckman Young Investigator Award. He is a fellow of the American Physical Society. In 2006 he was elected a member of the National Academy of Sciences, USA and in 2009 fellow of the American Academy of Arts and Sciences and of the Brazilian Academy of Sciences. In 2011 he was awarded the Einstein Professorship by the Chinese Academy of Sciences (CAS) and in 2012 he has been elected Fellow of the Biophysical Society. In 2014 he received the Diaspora Prize from the Ministry of Foreign Affairs and the Ministry of Industrial Development and Foreign Trade from Brazil.

Osvaldo Cori Lecture

REDOX CONTROL OF CALCIUM SIGNALING MEDIATED BY RYANODINE RECEPTOR CHANNELS: ITS EFFECTS IN HEALTH, AGING AND DISEASE

Hidalgo C[1], [1]BNI, CEMC, Programa Fisiol. Biofis., Medicina, Universidad de Chile. (Sponsored by Supported By BNI-09-015F; FONDECYT 1140545.)

Calcium release mediated by ryanodine receptor (RyR) channels plays key roles in calcium signaling in diverse cell types. In particular, an increase in intracellular calcium concentration stimulates RyR channel activity giving rise to calcium-induced calcium release (CICR), a signaling mechanism essential for cardiac muscle contraction and other cellular responses. In our group, we have shown that redox modifications of specific RyR cysteine residues, including *S*-glutathionylation and *S*-nitrosylation, determine RyR-mediated CICR. Thus, reactive oxygen and nitrogen species (ROS/RNS) promote RyR-mediated CICR whereas reducing agents exert the opposite effect. Results will be presented showing a central role for redox-sensitive RyR-mediated calcium release in insulin secretion from pancreatic beta-cells, and skeletal and cardiac muscle function. In addition, recent findings from our group indicate that hippocampal synaptic plasticity and memory processes require RyR-mediated calcium release. Moreover, aging and Alzheimer's disease - two conditions that result in increased neuronal oxidative tone - promote anomalous RyR-mediated calcium release, which presumably contributes to the defective neuronal function associated to these conditions. Altogether, these findings place redox-sensitive RyR-mediated calcium release as a central mechanism for diverse cellular functions, and implicate malfunctioning RyR-mediated calcium signaling in Alzheimer's disease or aged-related memory deficits.

Dr. M. Cecilia Hidalgo T. studied Biochemistry at the Universidad de Chile and graduated in 1965 after finishing her thesis work on the bioenergetics underlying epithelial sodium transport under the direction of the late Dr. Mitzy Canessa. She obtained her doctoral degree in 1969 as the first graduate of the Faculty of Sciences, Universidad de Chile, after working on squid axon physiology in Montemar, Chile. After a postdoctoral stay at the National Institutes of Health, Bethesda, MD, USA (1969 – 1972) working in molecular biology, she joined the Faculty of Chemical Sciences and the Faculty of Medicine at the Universidad de Chile, Santiago Chile (1972-1975), where she resumed the research on calcium signalling initiated in Montemar with Dr. Eduardo Rojas. She moved to the Dept. of Muscle Research Boston Biomedical Research Institute in 1974, where she continued working on calcium signalling and soon became Staff Scientist. She returned to Chile in October 1983, and she is currently Full Professor, Faculty of Medicine, Universidad de Chile. The current focus of her lab is the redox regulation of calcium release mediated by Ryanodine receptors channels from muscle, brain and endocrine cells. She has 114 ISI publications (4940 citations, h-index 43; Google scholar).

Severo Ochoa Lecture

NETWORKS OF ALTERNATIVE SPLICING REGULATION IN CANCER

Valcárcel, J. ICREA and Center for Genomic Regulation (CRG), Barcelona

In contrast with bacterial genes, which can be directly translated into proteins after transcription, genes in higher eukaryotes display a strange syntax whereby primary transcripts contain apparently irrelevant sequences (introns) that need to be removed to splice together the meaningful parts of our genetic messages (exons). To allow the splicing process, our cells have evolved one of the most complex molecular machineries, the spliceosome, that chaperones introns into conformations that exploit the catalytic properties of RNA by precisely the same chemical mechanisms used by self-catalytic introns.

Alternative splicing regulates the vast majority of human genes by generating alternative mRNAs that encode proteins with distinct, sometimes antagonistic functions. Alterations in alternative splicing can impact every hallmark of cancer and can be caused by cancer-associated mutations in splicing regulatory sequences or in splicing factors. In my talk, I will describe our recent efforts to systematically reveal splicing regulatory circuits altered in cancer cells and the potential use of this knowledge to design anti-cancer therapies.

These include methods for saturation mutagenesis of alternative exons, genome-wide identification of regulatory factors, and reconstruction of splicing regulatory networks via profiling of alternative splicing after systematic knock down of spliceosomal components. Our results reveal high dense regulatory content of alternative exon sequences and extensive regulatory potential of core splicing factors. They also reveal detailed molecular mechanisms of versatile splicing modulation by anti-tumor drugs and modified antisense oligonucleotides, as well as by signaling pathways important for cancer cell proliferation.

Dr. Juan Valcárcel obtained his PhD from work on influenza virus splicing regulation carried out in the lab of Juan Ortín at the Center of Molecular Biology Severo Ochoa in Madrid. After a postdoc with Michael Green at the University of Massachusetts, working on mechanisms of 3' splice site recognition and regulation, he established his group at the European Molecular Biology Laboratory (EMBL) in Heidelberg in 1996. In 2002 the group moved to the newly created Center for Genomic Regulation (CRG) of Barcelona, where he is currently chair or the Gene Regulation, Stem Cells and Cancer Program and ICREA Research Professor as well as associated Professor at the University Pompeu Fabra. Elected EMBO member in 2004, he was deputy Coordinator of the European Alternative Splicing Network of Excellence (EURASNET, led by Reinhard Lührmann), and head of the RNAREG Consolider consortium (which coordinated work of RNA biologists and molecular oncologists in Spain). He is also member of Scientific Advisory Board of Research Institutes and Companies from Spain, Portugal, France and USA as well as Editorial Board member of several journals including the RNA Journal, Molecular and Cellular Biology and Molecular Cell. He serves as reviewer for numerous journals and grant agencies, including the Board of Reviewing Editors of eLife and the Wellcome Trust interview panel. He has been recently appointed President of the RNA Society for 2017-2018. Work in his group focuses on molecular mechanisms and networks of alternative splicing regulation in cancer and pluripotent cells.

PABMB Lecture

DANGEROUS RELATIONSHIPS OF AN OLD ONCOGENE: RAS, WITH A SOUTH AMERICAN TUMOR SUPPRESSOR GENE: KLF6 WHO IS THE WINNER ?

Bocco, J.L. Centro de Investigaciones en Bioquímica Clínica e Inmunología CIBICI-CONICET, Dpto. de Bioquímica Clínica. Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. Córdoba – Argentina

ABSTRACT

KLF6 protein is a member of the Krüppel-like factors family of transcription factors which have diverse roles in the regulation of cell physiology including proliferation, apoptosis, differentiation and development. KLF6 is ubiquitously expressed and mutations within the *klf6* gene, decreased expression and/or loss-of-heterozygosity were associated with the development of different human malignancies.

We investigated whether KLF6 function could regulate oncogenesis triggered by the Ras pathway, considering that gain-of-function mutations of Ras are associated with more than 30 % of human cancers. We demonstrate that KLF6 behaves as a tumor suppressor, restraining the spontaneous onset of the transformed phenotype and reducing cell proliferation rate and tumor growth driven by mutated H-Ras. Depletion of KLF6 by *sh*RNA resulted in transformed foci formation and led to spontaneous conversion of NIH3T3 cells to a tumorigenic state. Moreover, ectopic KLF6 expression induced a G1-phase cell cycle arrest, thereby decreasing cell proliferation rate that was in line with increased expression of p21, whereas p21 knockdown impaired KLF6-induced cell cycle arrest. This cytostatic response was, however, associated with resistance to apoptosis mediated by DNA damaging chemotherapy drugs, though senesce induction cannot be excluded. Thus, constitutive KLF6 expression reverted the capabilities of Ras-transformed cells for growing in density- and anchorage-independent manner. Furthermore, xenotransplanted tumors in mice shown delayed onset and decreased growing ability when mice were challenged with cells stably expressing KLF6.

These findings provide novel evidence highlighting KLF6 function in response to oncogenic stress, suggesting a relevant activity of KLF6 in controlling cell proliferation and hindering tumorigenesis.

Dr. Jose Luis Bocco, born in Córdoba province, Argentina, was graduated in Biochemistry in 1983 at the Faculty of Chemical Sciences of the National University of Cordoba. He performed his doctoral studies in the same Institution, at the Department of Clinical Biochemistry, nowadays also CIBICI[#], and obtained his Ph.D. degree in 1989 working in biochemistry and regulation of gene expression. Between 1990 and 1993 he obtained a fellowship from the Association pour la *Recherche contre le Cancer* (Association for Cancer Research) to perform a post-doctoral training in Strasbourg, France, working on transcriptional regulation of gene expression by viral oncoproteins. Since 1994, he is a CONICET Researcher, currently belonging to the "Superior Investigator" category. Between 2000 and 2009 he was designated Associate Professor (Temporary Service) in the Louis Pasteur University, Strasbourg, France, participating in teaching activities at the École Supériuere de Biotechnologie de Strasbourg. Also since 1994, he is a Professor at the *Departamento de Bioquímica Clínica* and his current position is Full Professor. Dr. Bocco has been the Director of seven doctoral thesis in the field of gene expression regulation and molecular microbiology, leading to the conformation of several independent groups in a variety of fields including oncogenesis, tumor suppressor genes, bacterial pathogenesis and molecular epidemiology of clinically relevant bacteria, yielding more than 50 peer-reviewed publications in good impact factor journals like *PNAS, Oncogene, FASEB Journal, The Journal of Biological Chemistry, Cell Death & Differentiation, Molecular and Cellular Biology, Journal of Clinical Microbiology, Mechanisms of Development*, *PLoS Genetics, PLoS One*, etc. His research work contributed to the discovery of a new transcription factor, KLF6 (Krüppel-like factor 6), providing mechanistic evidence about the biology of KLF6 as a tumor suppressor. Since 2012 Dr. Bocco is a member of the Group of Investigators of BIOHEMO, an Applied Research Project, involving CONICET and "Laboratorio de Hemoderivados" - Universidad Nacional de Córdoba (Laboratory of Blood-derived products, National University of Cordoba), for the production of the human coagulation factor IX thorough recombinant technology. Since 2014 Dr. Bocco is the Principal Investigator of the multidisciplinary project PAE-GSK, funded by the GlaxoSmithKline company and MINCyT, which is aimed to identify new targets for anti-cancer therapy through a strategy based on synthetic lethality. From 2010 to 2015, and again from 2015 to 2020, he was designated as Chairman of CIBICI, position obtained by public contest in both opportunities. Between 2013 and 2015, he was designated Director of the *Centro Científico Tecnológico* CONICET Córdoba (CCT-Cordoba) by peer votation among Directors of all the Research Institutes belonging to CONICET in the Córdoba region, which includes more than 1000 researchers.

Symposia 1

Structural and Functional Characterization of Macromolecular Complexes

Chair: Nelson Barrera, Pontificia Universidad Católica de Chile

Visualizing activation-induced structural changes in ionotropic glutamate receptors using fast-scan AFM

Edwardson, J. M., Department of Pharmacology, University of Cambridge, U.K. (jme1000@cam.ac.uk)

Glutamate is the major excitatory neurotransmitter in the mammalian brain. It binds to three types of tetrameric ionotropic glutamate receptors (iGluRs): AMPA, kainate and NMDA receptors, and generates synaptic currents crucial to brain function. Several iGluR crystal and cryo-EM structures have been reported, which provide 'snap-shots' of the receptors as they transition between different states. What is lacking is information about the kinetics underlying these transitions. I will describe the use of fast-scan atomic force microscopy (AFM) to study activation-induced structural changes in native, full-length iGluRs, integrated into supported lipid bilayers.

Activation triggers a ~1-nm reduction in the height of the extracellular domain of all three types of iGluRs. Pharmacological and mutational manipulations indicate that this height reduction represents entry into the desensitized state. By scanning the AFM tip repeatedly back and forth over individual kainate receptors while caged glutamate was photolysed, we showed that the activation-induced height reduction occurs with half-time of ~0.8 s.

The kainate receptor appears as a double blob, with each blob representing a pair of N-terminal domains (NTDs). We found that the relative mobility of the NTDs becomes greater after stimulation. Intriguingly, some activation traces show switching between two mobilities, consistent with the existence of two states of the receptor that have different degrees of engagement between the NTDs. We suggest that we are looking at cycles of disengagement and re-engagement of the NTDs that may be preparing the receptor for successive rounds of activation, and which may be electrically silent.

Funding: Wellcome-Trust-grant 089125; BBSRC-grant-BB/J018236/1.

Dynamic protein structure: from protein disorder to membrane pores

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Our work focuses on aspects of dynamic and heterogeneous protein conformations and assemblies, using an integrated structural approach based on “native” mass spectrometry, ion mobility, and surface mapping techniques in combination with electron microscopy, SAXS and other biophysical methods. We will briefly introduce the different mass spectrometry-based Structural Proteomics approaches, and highlight the type of data which they can generate, and how they can be integrated with other structural information and with computational models.
Specifically, we are going to show recent results on the detection and characterization of intrinsic disorder in proteins, including alpha-synuclein and the apoptosis-related BAX protein. A range of folding states, from disordered to compact, are characterized and interpreted using molecular dynamics approaches. These data link the conformational state of the protein with their association into larger oligomers, which are believed to be able to form membrane pores. We use detergent micelles, lipid bilayers (bicelles) and nanodiscs for both native MS and covalent labelling of exposed parts of the protein, and apply these techniques to various different ion channels including the mechanosensitive channel of large conductance (MscL). Using covalently attached, charged ligands inside the MscL channel, we can mimic the effect of mechanical pressure on the surrounding membrane and characterize various opening states using ion mobility-MS, electron microscopy, EPR spectroscopy and other biochemical and computational methods, in the absence of lipids.

Single molecule pharmacology of P2X receptors

Barrera, N.P. Department of Physiology, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Chile. (nbarrera@bio.puc.cl)

In this symposium, I will discuss a novel application based on Atomic Force Microscopy (AFM) to characterize the agonist/receptor binding process at the single molecule level. By using purified P2X receptors inserted in artificial lipid bilayers and AFM tips functionalized with ATP analogues, it was possible to detect interaction forces between the agonists and single receptors that can be extrapolated to Kd values. These data can be correlated with whole-cell electrophysiological recordings of P2X receptors expressed in HEK cells. Combining docking experiments of the agonist binding along with Mass Spectrometry (MS) measurements of the agonist three-dimensional orientation, it can be possible to estimate the free energy of the agonist-receptor binding event, and in consequence to determine the conformational changes associated to the receptor activation. Two agonists, azido-ATP and EDA-ATP were pharmacologically evaluated onto P2X2 receptors. These experiments have provided a theoretical and experimental proof of principle for the use of AFM on pharmacology of membrane receptors at the single molecule level. It is anticipated that this methodology applied to novel receptors, combined with drug rational design, will provide a new tool to explore heterogeneous populations of membrane receptors in living cells. Funded by Fondecyt 1120169, DPI-20140080 and Millennium Science Initiative P10-035F grants.

Modulating TRPV1's gating with genetically encoded cross linkers.

C.K. Colenso[1], D. Cabezas[1], Juan C. Opazo[2], D. Granata[3], V. Carnevale[3], C.A. Ahern[4] and **S. Brauchi[1]** (sbrauchi@uach.cl)

1. Instituto de Fisiologia and Center for Interdisciplinary Studies on Nervous system (CISNe), Universidad Austral de Chile. 2. Instituto de Instituto de Ciencias Ambientales y Evolutivas, Universidad Austral de Chile. 3. Institute for Computational Molecular Science, Temple University. 4. Molecular Physiology and Biophysics, University of Iowa.

Membrane voltage, ligand binding, mechanical force and temperature can all induce conformational changes that open ion channel pores. A key question in understanding ion channel function is how the protein domains involved in sensing stimuli communicate with the pore to modulate ion channel's gate. Transient Receptor Potential (TRP) proteins are a large family of polymodal cation-permeating ion channels associated to cellular sensing of the environmental conditions. Although great advances have been made regarding the activation and modulation of TRP channel activity, detailed molecular mechanisms governing TRP channel gating are still needed. Sensitive to electric, chemical, mechanical and thermal cues, the temperature-activated TRPV channels are tightly associated with the detection and integration of *sensory input* of different nature, emerging as a model to study the inner-workings of polymodal activation. In this context, the intracellular helix dubbed TRP domain (TD) has been suggested as an integrator of input stimuli in TRP channels, however, the mechanics of such integration is currently unknown. Here we show an extensive bioinformatic analysis for bona fide TRP channels from unicellular eukaryotes and higher organisms highlighting important interactions connecting the TD with other portions of the channel. Patch clamp recordings performed on HEK-293T cells expressing TRPV1 channels containing genetically encoded cross linkers allowed us both to trap different conformations of the channel and to follow agonist-dependent motion of TD. We propose the first mechanistic model for TRP domain-associated integration of the input signals.

Dr. Mike Edwardson: Dr. Edwardson graduated as Bachelor in Natural Sciences in 1976 and received his PhD degree in Pharmacology in 1979 at the University of Cambridge, UK. He then worked for a short time as a post-doctoral research associate with Prof. Alan Cuthbert, FRS, before moving in 1980 to a Lectureship at the School of Pharmacy, University of London. He returned to Cambridge in 1984 to a Lectureship. He was subsequently promoted to Reader in 2000 and became Professor of Molecular Pharmacology in 2009. He is Fellow and Director of Pre-Clinical Studies at Christ's College and he is currently Head of the Department of Pharmacology at the University of Cambridge. His research interests deal with the imaging of biomolecules using atomic force microscopy. This tool is applied in his current research projects which involve the activation-induced structural changes in ionotropic receptors, the interaction of the sigma-1 receptor with ionotropic receptors and ion channels, the mechanism underlying the interaction of urinary exosomes with the primary cilium, the structure and behavior of synaptotagmin, interactions of proteins with lipid bilayers and the protection of teeth against dental caries.

Dr. Frank Sobott: Dr. Frank Sobott received his Ph.D. degree at the University of Frankfurt, Germany, at the Institute for Physical and Theoretical Chemistry, working in the Characterization and Application of Laser desorption mass spectrometry, supervised by Prof. Bernhard Brutschy and Prof. Michael Karas. From 2000- 2004 he was a Post-doctoral research associate at the laboratory of Prof. Carol V. Robinson FRS, at Cambridge and Oxford Universities, U.K. There, he specialized in Mass Spectrometry applied to large macromolecular complexes. In 2004 he became Principal Investigator at the Department of Biochemistry, University of Oxford, U.K, and later, in 2009, he moved to Belgium and joined the University of Antwerp, as Assistant Professor. The focus of his research is on the analysis of non-covalent interactions in supra-molecular systems and large functional assemblies of biomolecules. His group is developing new methods and instrumentation for the analysis of multi-component, heterogeneous and dynamic assemblies based on mass spectrometry and ion mobility spectrometry and associated techniques.

Dr. Nelson Barrera: Dr. Barrera started his academic career as a Biochemist from the Pontificia Universidad Católica de Chile, in 1997. In this institution he also received the Master Degree in Biochemistry (1999), and the PhD degree in Biological Sciences, mention in Phisiology, in 2004. During those years he worked with Professor Manuel Villalón on the beating frequency of ciliated cells and, the signaling transduction pathways underlying the process. In 2004 he moved to Cambridge for his postdoctoral training with Dr. Mike Edwardson at Cambridge University. His research focused on ionotropic receptors, particularly in the use of Atomic Force Imaging to determine its stoichiometry and spatial subunit arrangement. In 2007, still at Cambridge, he started working with Dr Carol V. Robinson. There he worked on the Mass Spectrometry analysis of membrane proteins. Since 2009 he is part of the Faculty of Biological Sciences at Pontificia Universidad Catolica de Chile as Assistant Professor. His current research is focused in protein biophysics and cell mechanical properties at the single molecule level.

Dr. Sebastian Brauchi: Dr. Brauchi graduated as Biochemist in 2001 from Pontificia Universidad Catolica de Valparaiso, Chile, working on olfactory physiology with Dr. Juan G. Reyes. In 2006 he got his Ph.D. in Sciences, mention in Molecular and Cellular Biology from Universidad Austral de Chile, working on protein biophysics of thermo TRP channels with Dr. Ramon Latorre. In 2008 Dr. Brauchi was awarded with a PEW Fellowship, and moved to Boston for his postdoctoral training with Dr. David E. Clapham at Boston Children's Hospital, Harvard Medical School. There, he studied the effect of TRPM7 conductance on synaptic vesicle fusion and the gating mechanisms of TRPM8 channels. Dr. Brauchi joined the Physiology Institute at Universidad Austral de Chile as an Investigator in 2008. His research group studies the activation and modulation of molecular sensors, the evolution of TRP ion channels proteins, and the biophysical properties of ion channels. His group is also interested in the development of novel experimental approaches including optical methods and platforms for image analysis.

Symposium 2

Functional Genomics of *P. salmonis*: *Unraveling the pathogenicity traits in the P. salmonis genome*

Chair: Alejandro Yañez, Universidad Austral de Chile

Discovering and comparing pathogenic mechanisms present in the P. salmonis strains genomes and proteomes".

Yáñez Cárcamo[1,2] A.J., Oliver, C. Haro, R., Sánchez, P. Cortes, M. Sandoval, R., Albornoz, R., Romero, A., Cárcamo, J.G., Enríquez, R., Avendaño-Herrera, R., Figueroa..[1] J. Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile[2]; Interdisciplinary Center for Aquaculture Research (INCAR), Concepción, Chile; [3]Universidad Andres Bello, Viña del Mar, Chile

Piscirickettsia salmonis is a facultative intracellular Gram-negative bacterium isolated from salmonids in Chile and constitutes one of the main problems in farmed salmonids. To study this bacterium, we have developed the first broth media named Austral SRS-Broth which CLSI included in the last guideline edition of drug susceptibility tests. We report the first genome sequence of a virulent strain, which was isolated from Rainbow trout (Oncorhynchus mykiss) and cultured in Austral SRS-Broth. The genome analisys and purifications of outer membrane vesicles (OMVs), let us to identify and study a variety of genes associated with pathogenicity, toxins, environmental adaptation, metabolic pathways, iron acquisition, secretions systems, expression levels of several multidrug resistance pumps as well as transposable elements and insertion sequences (ISs). The complexity of the *P. salmonis* genome was compared with a proteomic analysis (LC-MS/MS) for Multidimensional Protein identification technology (MudPit). These data base at different biological scales add new information of *P. Salmonis* and provide the framework for development of specific PCR-based diagnostic tools, reverse vaccinology and systems biology approaches for discovery of therapeutic targets. FONDAP-INCAR 15110027.

Dissecting the pathogenesis of Piscirickettsia salmonis by mutational analysis

Mancilla, M., Saavedra, J. & Hernandez, N.
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Piscirickettsiosis is by far the most important infectious disease affecting the Chilean salmon industry. Its impact does not only result from economic losses caused by mortality, but also from the environmental challenge that arises because large amounts of antibiotics are necessary to control the frequent outbreaks. The etiologic agent *Piscirickettsia salmonis* is a Gram-negative, intracellular facultative, slow-growing and nutritionally demanding bacterium, whose pathogenic nature remains poorly understood. An important step towards a deeper understanding of its pathogenicity, and thus the development of biotechnological products that allow for a better disease control, is the establishment of a protocol for genetic manipulation. In this work, we provide a detailed description of the steps realized to construct the first *P. salmonis* mutant. By means of plasmid cloning and mobilization to an appropriate *P. salmonis* receptor strain, we accomplished the stable integration of the construct in its chromosome, disrupting a gene putatively required for lipopolysaccharide (LPS) biosynthesis. Phenotypic analysis suggested that the mutant carried a defect in the outer membrane, which may correlate with an expected reduction in virulence. To test this hypothesis, further *in vitro* analyses were conducted in the CHSE-214 cell line.

Altogether, our results demonstrate that the genetic manipulation of *P. salmonis* is feasible, and that the LPS plays a major role in host-pathogen interactions.

Funding: CORFO grant 15ITE1-45434.

The landscape of non-coding RNAs in Piscirickettsia salmonis

Vinicius Maracaja-Coutinho[1,2,3], Cristian Molina[4,5], Marcelo Rojas-Herrera[4,5], Guillermo Nourdin-Galindo[5], Raúl Arias-Carrasco[1], **Alejandro J. Yañez**[4,5]
[1]Universidad Mayor, Santiago, Chile. [2]Instituto Vandique, João Pessoa, Brazil. [3]Beagle Bioinformatics, Santiago, Chile. [4]Universidad Austral de Chile, Valdivia, Chile. [5]Austral Omics, Valdivia, Chile.

Piscirickettsia salmonis is a facultative intracellular bacteria, Gram-negative and the etiologic agent of Piscirickettsiosis (SRS). It is highly aggressive, responsible to a mortality of up to 90% of infected salmons, affecting drastically Chilean and worldwide aquaculture. Besides the existence of nine genome sequences from different isolates in public databases, the mechanisms behind P. salmonis pathogenicity remains unclear. Non-coding RNAs (ncRNAs) are transcribed molecules not translated into proteins. In Bacteria, these molecules are known to be involved in the fine-tuning regulation of different cellular mechanisms, including iron homeostasis, cell cycle, quorum sensing, secretion systems and secreted virulence factors. Here, we applied two in-house bioinformatics strategies, based on (i) RNA secondary structure and (ii) primary sequences analysis, in order to identify the repertoire of ncRNAs on the genome of nine P. salmonis strains. Our analysis revealed a set of 393 to 563 ncRNAs spreaded over all isolates, including RNAs associated with virulence regulation, and others with similar characteristics of microRNAs and piwiRNAs. Predicted RNAs had their transcription validated using RNA-seq, and their conservation analyzed within P. salmonis isolates and organisms from other domains of life. These molecules may have critical roles in P. salmonis biology, modulating important cellular mechanisms, including fish pathogenesis. All data generated was stored in a public domain database, named PsalmonisDB. This is the first comprehensive characterization of the repertoire of ncRNAs in different P. salmonis isolates, revealing specific and shared RNAs between isolates and other organisms, opening new avenues to understanding the molecular mechanisms behind fish pathogenesis.

Research on Iron acquisition of *Piscirickettsia salmonis*

Segovia[1] **C. and Santander**[1,2] **J.**
[1]Universidad Mayor, Center for Genomis and Bioinformatics, Huechuraba, Chile
[2]Memorial University of Newfoundland, Department of Ocean Sciences, St John´s, Canada

Piscirickettsia salmonis is a fastidious intracellular facultative Gram-negative bacterial pathogen causing the Salmonid Rickettsial Septicemia (SRS). *P. salmonis* biology is not well understood, including its molecular mechanisms of pathogenesis. Host vertebrates sequester iron from invading pathogens as a means of nutritional immunity using high-affinity iron-binding proteins. Invading bacterial pathogens sense this iron depletion as a signal that they are within a host and up- and down- regulates expression of genes to overcome the host defenses. Here we will describe three aspects of the *P. salmonis* iron metabolism. First, we describe a functional Fur protein by using a heterologous system. Second, we describe the up- and down-regulate genes under iron deprivation conditions in *P. salmonis* mediated RNA-Seq analysis and the possible Fur binding box. Among 161 differentially expressed transcripts (fold change > 2), 67 were down-regulated and 94 up-regulated under iron-limited conditions. We identified down-regulated genes related to type IV, VI secretion systems and ABC transporters, DNA remodeling proteins and hypothetic genes that could be related to host entrance invasion. Up-regulated genes are related to DNA binding proteins, membrane stress response, membrane transporters, flagellar and chemotaxis proteins and several unique genes encoding hypothetic proteins that could be regulated in a Fur-dependent fashion. We identify an inner membrane ferric transport system, but the precise mechanism of iron acquisition during pathogenesis, which likely is related to unique putative *P. salmonis* genes, could not be identified. Finally, here we describe ncRNA iron up- and down-regulated that could play a role during pathogenesis. The present study provides relevant genomic information about *P. salmonis* pathogenesis.

Dr. Marcos Mancilla studied Biochemistry at the Universidad Austral de Chile, Valdivia, Chile. In 2008, he obtained his Ph.D. in Molecular and Cell Biology from the same university studying Brucella abortus genomic islands, the etiologic agent of bovine brucellosis. After a postdoctoral stay at the Universidad de Navarra, Pamplona, Spain and intense research on genetics of B. abortus lipopolysaccharide (2009 – 2011), he returned to Chile to join the Institute for Biochemistry and Microbiology at the Universidad Austral de Chile (2012 – 2013), where he was employed as a Research Associate to improve diagnostics of bovine tuberculosis. In 2014, he moved to ADL Diagnostic Chile Ltd, a diagnostic and biotechnology company based in Puerto Montt, Chile, where he is currently holding the position of Research Director. The focus of Mancilla's Lab is on pathogenesis of bacterial salmonid diseases. The group pioneered a method for genetic manipulation of Piscirickettsia salmonis, and recently obtained the first mutant strains. He will present this work in the conference under the title "Dissecting the pathogenesis of Piscirickettsia salmonis by mutational analysis".

Dr. Alejandro Yáñez Carcamo obtained his bachelor degree and professional title of Biochemistry at Universidad Austral de Chile. Also he got his PhD degree in Sciences, Molecular and Cell Biology in the same university. He works as a Research Associate at Dr. Marino Martinez-Carrion lab´s in the University of Missouri-Kansas City. In 1999, Dr. Yañez became Associated Professor at Institute of Biochemistry in Universidad Austral de Chile and in 2011 became Full Professor. Actually, he is the head of *AUSTRAL omics* the first core facility in the south of Chile. The team of Yañez group is divided into two main areas: 1) Investigation of a possible treatment for diabetic nephropathy and 2) Study of the pathogens involved in the pathology of the most important fish diseases affecting our national aquaculture. In the latter area, Yañez team has been able to generate a novel broth medium for the culture of the fish pathogen *Piscirickettsia salmonis* and elucidate the main features of these bacteria genome. He will present the conference titled "Discovering and comparing pathogenic mechanisms present in the *P. salmonis* strains genomes and proteomes".

Symposium 3

Molecular basis of Alzheimer's disease

Chair: Victor Bustos, Rockefeller University, USA

Wnt-5a signaling and Alzheimer´s disease.

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Previous studies in our laboratory, suggest a relationship between the Wnt signaling pathway and the Alzheimer's disease (AD), in particular, at the level of the canonical Wnt pathway. In addition, we have described several synaptic effects of Wnt-5a, a ligand that in hippocampal neurons induce the activation of the non-canonical Wnt pathway. Activation of Wnt-5a signaling regulates synaptic structure and function in hippocampal excitatory neurons, promoting the PSD-95 clustering, development of dendritic spine morphogenesis, as well as, the increases in glutamatergic neurotransmission. More recently, we described that Wnt-5a regulated the expression of several microRNAs that could be involved in such synaptic effects. Interestingly, this ligand prevents the neurotoxicity induced by amyloid-b (Ab) aggregates, in a β-catenin independent manner. In this symposia, we present our work on the effects of Wnt-5a signaling in the nervous system, as well as, the evidences and hypotheses that point to a neuroprotective potential of the non-canonical Wnt pathway as a therapeutic control of AD.

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Autophagosomes cooperate in the degradation of intracellular C-terminal fragments of the Amyloid Precursor Protein via the MVB/lysosomal pathway: Dysregulation of amphisome biogenesis in Alzheimer's disease?

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Introduction: Brain regions affected by Alzheimer's disease display well-recognized early neuropathological features in the endo-lysosomal and autophagy systems of neurons, including enlargement of endosomal compartments, progressive accumulation of autophagic vacuoles, and lysosomal dysfunction. Although the primary causes of these disturbances are poorly understood, a growing body of evidence suggests that the amyloid precursor protein (APP) intracellular C-terminal fragment-b (C99), generated by cleavage of APP by BACE1, is the earliest initiator of synaptic plasticity and long-term memory impairment. The aim of this study was to evaluate the possible relationship between the endo-lysosomal protein degradation pathway and autophagy on proteolytic processing and C99 turnover. Methodology: Different methods oriented to perturb autophagosome formation or fusion of autophagosomes to endo-lysosomal compartments were used to evaluate the role of autophagy on C99 levels. Results and Conclusions: Herein, we show that inhibition of autophagosome formation, or blockage of the fusion of autophagosomes to endo-lysosomal compartments, caused massive accumulation of C99 in the lumen of endo-lysosomal organelles. In contrast, activation of autophagosome formation, either by starvation or inhibition of the mammalian target of rapamycin, enhanced lysosomal clearance of C99. Altogether, our results indicate that autophagosomes are key organelles for maintaining C99 clearance via the lysosomal pathway.

Role of glutamatergic synaptic transmission in Alzheimer's disease.

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Amyloid-β (Aβ) oligomers have been recognized to be early and key intermediates in Alzheimer's disease (AD)-related synaptic dysfunction. Aβ oligomers block long-term potentiation (LTP), a cellular model of learning and memory, and impair spatial memory through a mechanism mediated by glutamatergic receptors and oxidative stress. Wnt signaling has a well-established role as a regulator of nervous system development, but its role in the maintenance and regulation of established synapses in the mature brain remains poorly understood. At excitatory glutamatergic synapses, NMDA (N-methyl-D-aspartate) receptors (NMDARs) have a fundamental role in synaptogenesis, synaptic plasticity, and learning and memory; however, it is not known what controls their number, composition and signaling pathways downstream of its activation. NMDARs control critical events in synaptic organization and synaptic plasticity. On the other hand, NMDARs over activation can promotes neuronal death in neuropathological conditions. NMDARs localization (synaptic or extrasynaptic) determines its contribution to synaptic plasticity process or synaptotoxicity events (or excitotoxicity). Wnt-5a specifically upregulates synaptic NMDAR currents, facilitating induction of LTP, this effect requires an increase in postsynaptic calcium and downstream effectors of the Wnt signaling pathway. On the other hand, elevated level of oxidative stress has been related to poor cognitive performance, diminished LTP and synaptic damage mediated by NMDARs over activation. The role of NMDARs distribution and the functional consequences in calcium homeostasis associated to synaptic dysfunction and neuronal death present in chronic and acute mental diseases is a general mechanism of toxicity relevant for a wide range of mental conditions.

Brain extrasynaptic NMDA receptors may play a prominent and triggering role during early and presymptomatic stages of Alzheimer´s disease.

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N-methyl-D-aspartate receptors (NMDARs) are fast acting glutamate-gated channels highly permeable to calcium ions which play an essential role in the well functioning and maintenance of synaptic plasticity in the brain as well as in the induction and transcription of gene programs related to neuronal survival and protection . Brain NMDARs also trigger intracellular signaling cascades leading to neuronal cell dysfunction and eventually to synaptic loss and cell death. Recent studies suggest that such functional dichotomy of NMDARs may depend on the cellular localization of these receptors at the dendrite : synaptic NMDARs exert neuroprotective and pro-survival actions and induce common forms of synaptic plasticity found in the brain (LTP and LTD) whereas extrasynaptic NMDARs may trigger opposite actions including pro-oxidative effects, changes in neuronal synchronization, mitochondrial dysfunction, and eventually cell death. Recent experimental evidences, originated from animal models and cultured neurons, suggest that synaptic dysfunction and cellular loss in neurodegenerative diseases such as Alzheimer (AD), Huntington (HD) and Parkinson (PD), may result from a shift in the balance of NMDARs signaling from the survival-promoting synaptic NMDARs toward extrasynaptic NMDARs that induce cell death pathways. We propose that an imbalance, in extrasynaptic versus synaptic NMDARs signaling, is an early and presymptomatic hallmark of these diseases . In this presentation, we will focus into the functional and pharmacological distinctions between synaptic and extrasynaptic NMDARs in relation to the vey early stages of AD.

Dr. Waldo Cerpa is an Assistant Professor of the Department of Molecular and Cellular biology at the Pontificia Universidad Catolica de Chile. His research focuses on the elucidation of the mechanisms by which the oxidative stress modulates the function of NMDA receptors. Dr. Cerpa studied biochemistry and neurobiology at the Pontificia Universidad Catolica de Chile. His thesis focused on the protective role of the Amyloid Precursor Protein against copper-induced neurotoxicity. He received his PhD in 2009, working on the regulation of NMDA-receptor synaptic transmission by the Wnt signaling, under the direction of Nibaldo Inestrosa. His postdoctoral training was in the group of Professor Andres Barria in the Department of Physiology and Biophysics at the University of Washington Seattle, where he identified RoR2 as a Wnt receptor that regulates synaptic NMDARs.
Dr. Patricia Burgos is an Assistant Professor of the Department of Physiology at the Universidad Austral de Chile. Her laboratory studies the crosstalk between endoplasmic reticulum-associated degradation and lysosomal function. Dr. Burgos received her bachelor's and master's degrees in biochemistry from the Universidad Austral de Chile. She received her PhD in Cell and Molecular biology from the Pontificia Universidad Catolica de Chile, where she studied the mechanisms of regionalization of proteins on the cell surface, under the direction of Alfonso Gonzalez. Her first postdoctoral position was at University of Colorado in Denver, in the laboratory of John Hutton, where she studied the trafficking of the dense-core vesicle membrane protein phogrin. Next, she held a postdoctoral position at the NIH in the laboratory of Juan Bonifacino, where she studied the trafficking of the Amyloid precursor protein with emphasis on the endocytic pathway. Dr. Burgos is a member of the Chilean Society for Cell Biology and the American Society for Cell Biology.

Dr Gonzalo Bustos is an emeritus professor at the Pontificia Universidad Catolica de Chile. He has authored more than 70 scientific articles, focusing on neuropharmacology and signal transduction in the brain. After obtaining his PhD in pharmacology from the University of Toronto, Dr. Bustos received postdoctoral training in the department of pharmacology in Yale University. His main research interests are the regulation of excitatory amino acid release and excitatory amino acid receptor function and expression in basal ganglia structures in the brain, the neurochemical adaptive changes of dopamine-and glutamate-neurons in experimental models of brain neurodegenerative disease, and the Interaction between glutamate-neurons and neurotrophic factors (BDNF and GDNF) in experimental models of Parkinson's disease. During his career, Dr. Bustos has held the position of Director, Vice-president and President of the Society of Pharmacology of Chile. Currently, Dr. Bustos is a professor in the PhD program of Chemical Sciences and Pharmacology at the Universidad de Chile and professor and coordinator of a program in neuropsicopharmacology in the Faculty of Medicine at the Universidad de Chile.

Dr. Juan Francisco Codocedo obtained his undergraduate degree in Biochemistry in 2007 from Universidad de Santiago de Chile studying the maturation of GABAergic currents in the visual cortex. After working as a research assistant at Dr. Huidobro's lab in Pontificia Universidad Católica de Chile, Dr. Codocedo started his doctoral studies in Molecular and Cellular Biology in the same institution, describing the role of microRNAs in the postsynaptic effect of Wnt signaling, under the direction of Dr. Nibaldo Inestrosa. Currently, he works as a Postdoctoral researcher in Dr. Inestrosa's lab, evaluating the effects of different compounds in the induction of synaptic plasticity in WT and Alzheimer's disease models.

Dr. Victor Bustos: Dr. Bustos is a senior research associate at the Rockefeller University in the laboratory of Paul Greengard. His research focuses on the cellular and molecular mechanisms which regulate Abeta levels in the brain. He completed his Bachelor degree at the Pontificia Universidad Catolica de Chile, where he did an undergraduate thesis under the direction of professor Gonzalo Bustos. He received his PhD in Biomedicine from the Universidad de Chile where he worked under the direction of professor Jorge Allende. His graduate research focused on revealing the mechanisms by which protein kinases recognize their substrates. He performed his postdoctoral training in the Venetian Institute for Molecular Medicine, before moving to the Rockefeller University.

Symposium 4

Effect of force in Biology: from enzymes to signaling in the cell

Chairs: **Lisette Leyton** and **Christian A.M. Wilson**, Universidad de Chile

DNA springs and the deformability of enzymes

Zocchi G[1], [1]Physics and Astronomy UCLA.

Enzymes are reversibly deformed by the binding and release of substrates and products. Conformational motion may be viewed as driven by the elastic energy transferred to the enzyme by chemical binding events. Over the past ten years we have developed the experimental means of artificially injecting elastic energy into enzymes in a controlled and addressable manner. Deforming the enzyme generally results in reversible modulation of activity: a generalized form of allosteric control. The method of the DNA molecular springs represents a new tool to probe the mechanics of the enzymatic cycle. I will illustrate these ideas through various experimental results obtained with our enzyme – DNA supramolecular constructions. These new molecules may also be developed into a new generation of molecular probes.

Mechanical and functional studies of biomolecules at single molecule level

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The effect of force on protein structure an associated changes of protein function is a subject of current intensive research. Mechanical forces are generated inside the cell during such diverse molecular processes as transcription, replication, translation, chromosomal segregation, protein unfolding, translocation of proteins across membranes, cell locomotions among others. Recent technological advances now allow the application and measurement of forces on biomolecules with extreme precision. In particular, the so-called “analytical optical and magnetic tweezers” instruments can manipulate single molecules, such as proteins and nucleic acids, while measuring their internal stress forces generated in the course of biological processes. In this study we used the optical tweezers (OT) to study the mechanical stability of adenylate kinase (AK) from the thermophilic organism Aquifex. AK was first characterized in OT and was found to unfold around 25 pN during force-extension experiments with a fast (mseg) 4 nm intermediate at 15 pN. This intermediate could correspond to the ATP binding domain unfolding independently of the rest of the protein. In another project we study the effect of the chaperon BiP in the folding and unfolding pathways of different protein. We found that BiP bind to the proteins in the unfolded state.

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The proteoglycan Syndecan-4 potentiates integrin-induced cell contraction.

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Astrocytes in a pro-inflammatory context are an impediment to axonal regeneration. Reactive astrocytes undergo changes in shape and surface protein expression. αvβ3 integrin, a receptor up regulated in reactive astrocytes, interacts directly with neuronal Thy-1 to suppress neurite outgrowth and induce retraction of existing neuronal processes; however, blocking αvβ3 integrin only partially prevents these events suggesting the participation of other molecules. We propose that Syndecan-4, another up regulated Thy-1 receptor, binds to Thy-1 promoting integrin-mediated effects in neurites. Using neurons seeded over astrocytes, and pharmacological, as well as genetic manipulations, we tested Syndecan-4 effect in astrocyte-dependent suppression of neurite outgrowth. Additionally, the effect of Syndecan-4 on neurite retraction induced by αvβ3 integrin was tested with differentiated neurons incubated with a combination of αvβ3-Fc and Syndecan-4-Fc proteins. Our results indicate that neurite outgrowth, suppressed upon contact with astrocytes, was induced when Thy-1-Syndecan-4 interaction was avoided by silencing of Syndecan-4. In addition, neurite outgrowth over astrocytes was enhanced even more when β3 integrin was also blocked. Alternatively, αvβ3-promoted neurite shortening was more rapid when co-incubated with Syndecan-4-Fc. Finally, using Optical tweezers-assisted dynamic force spectroscopy, a direct interaction between Syndecan-4 and Thy-1 was demonstrated and quantitative information characterizing the free-energy landscape of the dissociation process was obtained. These findings indicate that the combined interaction of the astrocyte αvβ3 integrin and Syndecan-4 with neuronal Thy-1 synergizes to suppress neurite outgrowth and induce contraction of existing processes. Thus, Syndecan-4 is a receptor in astrocytes that contributes to the non-permissive environment for axon regeneration.

Cells feel the force… then they don't

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Cells, particularly stromal cells, have highly evolved machinery to interpret physical forces. This machinery allows cells to mechanically probe the physical properties of their microenvironment, such as the elasticity and stiffness of the surrounding extracellular matrix (ECM). The physical properties of the ECM microenvironment may then drive specific cellular outcomes; the most immediate outcome being alterations in the cytoskeleton that result in a "matching" of the compliance of the cell and the ECM. Yet, the ECM is constantly being rebuilt and modified by the cell, leading to a system of dynamic reciprocity whereby cells and their ECM reach a type of mechanical homeostasis that some believe is critical to tissue homeostasis. For example, recent evidence suggests that aberrant increases in ECM stiffness associated with fibrosis sustain pathological progression by "activating" the stromal cell population to produce more scar/fibrosis through mechanisms involving cytoskeletal adaptations to the environment. In this talk, I will describe recent work in our lab that has identified a stromal cell subpopulation based on surface explression of the small GPI-linked glycoprotein Thy-1, that emerges during lung fibrosis that is agnostic to the mechanics of its microenvironment. The identification of the subpopulation led to the discovery of a novel regulatory mechanism by which Thy-1 regulates stromal cell mechanotransduction at the level of focal adhesion structures. Disruption of Thy-1-medated focal adhesion signaling leads to stromal cells that are inappropriately activated on/in homeostatic, "soft" ECM microenvironments and may be one of the earliest precipitating events in the initiation of tissue fibrosis.

Dr. Giovanni Zocchi studied undergraduate Physics at the Universita' di Pisa and Scuola Normale Superiore in Italy. He obtained his Ph. D. in Physics from the University of Chicago in 1990, working on nonlinear dynamics and turbulence under the direction of Albert Libchaber. After a postdoctoral stay at the Ecole Normale Superieure in Paris, France (1990 – 1993) he joined the Niels Bohr Institute in Copenhagen, Denmark (1994 – 1999), where he changed his field of research to biological physics. He moved to the Dept. of Physics and Astronomy at UCLA in 1999, where he is currently Full Professor. The focus of the Zocchi Lab is mechano-chemical coupling in enzymes. The group pioneered the artificial mechanical control of enzymes, and recently obtained the first experimental demonstration that enzyme conformational dynamics is viscoelastic.

Dr. Christian A.M. Wilson was trained as a Biochemist and obtained his Ph.D. from the University of Chile, Chile in 2011. CW permorfed a posdoctoral training at University of California, Berkeley, USA with Dr. Carlos Bustamante and Dr. Susan Marqusee (2011-2013). He then joined the Faculty of Chemistry and Pharmaceutical Sciences at the University of Chile in 2013, where he is currently an Assistent Professor at the Biochemistry and Molecular Biology department. One topic of his group is focused in determining the importance of the force associated to the domain movements of different protein to perform their function. Dr. Wilson lab has assembled the first optical tweezers instrument to measure force in individual molecules in the country.

Dr. Lisette Leyton studied Biochemist and obtained her Ph.D. from the University of Chile, Chile in 1990. In her doctoral thesis project, she described a sperm receptor for the zona pellucida, which upon interaction lead to the acrosome reaction. She then performed a postdoctoral training at Duke University, Durham, USA with Dr Patricia Saling (1990-1994) and another Postdoc at the Institute of Biochimie at Lausanne, Switzerland with Dr Claude Bron (1994-1998) studying signaling mechanisms involved in T cell activation. She then joined the Faculty of Medicine at the University of Chile in 1999, where she is currently Full Professor at the Cell and Molecular Biology Program. Dr. Leyton is currently studying the bi-directional communication that exists between neurons and astrocytes and how these cell-cell interactions increase astrocyte adhesion and migration as well as induce shortening of dendrites and axons in neurons under inflammatory processes.

Dr. Thomas Barker was trained in Chemistry, Physics and Biomedical Engineering and obtained his Ph.D. on the topic of cell cytoskeletal signaling from the University of Alabama at Birmingham, USA in 2003. He performed his postdoctoral training at the University of Washington and Hope Heart Institute, Seattle WA, USA with Dr. Helene Sage exploring mechanisms of action of the matricellular protein SPARC in regulating cell contractility and at École Polytechnique Federal de Lausanne, Switzerland with Dr. Jeffrey Hubbell developing engineering extracellular matrices to guide cell differentiation. He joined the faculty at Georgia Institute of Technology in 2006 and now holds the post of Associate Professor. Dr. Barker currently studies mechanisms of cell-ECM mechanotransduction in tissue homeostasis and fibrosis.

Symposium 5

Photobiology: from gene expression to optogenetics

Chairs: Claudia Stange, Universidad de Chile and **Luis Larrondo**, Pontificia Universidad Católica

Colors in the shade: molecular regulation of carotenoid biosynthesis by phytochrome-dependent pathways

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Plants use light as a source of both energy (for photosynthesis) and environmental information (via photoreceptors such as phytochromes). When the energy of the incoming light exceeds the photosynthetic capacity of plants, photoprotectants such as carotenoid pigments dissipate the excess of energy as heat and quench highly reactive oxygen species that might form. The production of carotenoids is repressed in the shade. Our recent work with *Arabidopsis thaliana* has unveiled a number of molecular factors involved in this process. We have shown that PIF1, a transcription factor of the phytochrome-interacting factor (PIF) family, down-regulates the accumulation of carotenoids by directly repressing the gene encoding phytoene synthase (PSY), the main rate-determining enzyme of the carotenoid pathway. PIF1 and other photolabile PIFs accumulate in the shade and degrade upon interaction with photoactivated forms of the photoreceptors phytochromes. We have also identified positive regulators of *PSY* gene expression, some of which also contribute to regulate carotenoid biosynthesis in Arabidopsis in response to shade signals. Interestingly, similar molecular mechanisms operate in tomato (*Solanum lycopersicum*) to modulate carotenoid biosynthesis during fruit ripening, when carotenoids accumulate to color the ripe fruit in red and hence signal that it is ready to be consumed (and the seeds dispersed). In this case, however, the shade signal is produced within the tissue after filtering of the light through the cell layers of the green fruit pericarp and it contributes to adjust carotenogenesis to the actual ripening stage. An integrated model summarizing our recent results will be presented.

Light affects plastid differentiation, carotenoid composition and gene profile in carrot roots

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Carotenoids are isoprenoid pigments responsible for yellow, orange and red colors found in nature. In plants, they are synthesized in plastids of photosynthetic and sink organs. They contribute to light-harvesting and photoprotection during photosynthesis, serve as scavengers for oxidative damage and are essential for phytohormone synthesis. Carotenoids also play important roles in human health acting as vitamin A precursors and antioxidants.

Carrot (*Daucus carota*) is the main source of dietary provitamin A and contrary to other plants, accumulates massive amounts of carotenoids (β-carotene and α-carotene) in the storage root that grows in darkness. Carotenoid accumulation and storage root development are in correlation with key carotenogenic gene expression, as phytoene synthase *DcPSY1* and *DcPSY2* expression. During seedling de-etiolation both genes are also induced showing a positive response to light in leaves. On the contrary, light has a profound effect during storage root development by inhibiting root thickening, affecting carotenoid accumulation and repressing the expression of most genes required for β-carotene biosynthesis. To find genes controlling high carotenoid accumulation we performed a RNA-Seq analysis of roots grown in dark and light conditions. Unexpectedly, we found several light-induced genes that correlate with carotenoid accumulation in the dark-grown root. The over representation of genes involved in photomorphogenesis such as *PAR 1* and photosynthesis (*PHYA*) let us to propose that carrot roots accumulate high levels of carotenoides because they have lost the ability to inhibit the de-etiolation and photomorphogenesis found normally in other roots.

Molecular responses to vegetation proximity or how to deal with competing neighbors

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In plants, perception of vegetation proximity by phytochrome photoreceptors activate a transcriptional network that implements a set of responses to adapt to plant competition, including elongation of stems or hypocotyls. In *Arabidopsis thaliana*, the homeodomain-leucine zipper (HD-Zip) transcription factor ATHB4 regulates this and other responses, such as leaf polarity. To better understand the shade regulatory transcriptional network, we have carried out structure-function analyses of ATHB4, which led us to conclude that this transcriptional regulator has two physically separated molecular activities: the HD-Zip, involved in binding to DNA-regulatory elements, and the ERF-associated amphiphilic repression- (EAR-) containing N-terminal region, involved in protein-protein interaction. Whereas both activities are required to regulate leaf polarity, DNA-binding activity is not required for the regulation of the seedling responses to plant proximity, which indicates that ATHB4 works as a transcriptional co-factor in the regulation of this response. It is currently known that a given transcription factor uses different mechanisms to regulate gene expression depending on the promoter context. Our results about the structure-function analyses of ATHB4, however, indicate that the same transcription factor uses distinct mechanisms to regulate even different physiological responses (cofactor activity for regulating the shade avoidance vs. transcription factor activity to regulate leaf polarity).

A Fungal-based Optogenetic Switch for Synthetic Biology and Art

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The fungus *Neurospora crassa* has been one of the main models for the study of photobiology. This ascomycete responds specifically to blue-light (but not to other wavelengths) through a transcriptional heterocomplex named White-Collar Complex (WCC). One of its components, WC-1, possesses a LOV (Light-Oxygen-Voltage) domain capable of detecting blue light, which promotes a conformational change that leads to a light-dependent dimerization that results in strong transcriptional activation. Thus, the expression of WCC-target genes is rapidly and precisely controlled in a dose-dependent manner when light is present. In order to design and improve optogenetic switches that can be utilized in other organisms as orthogonal controllers, we have been exploring the dynamics of light responses in this organism. Through the development of simple synthetic switches we have successfully implemented a blue-light responding transcriptional system in *Saccharomyces cerevisiae*. Therefore, now in yeast (which naturally does not respond to light) we can efficiently and orthogonally induce gene expression and control industrial phenotypes. In addition, in order to better identify the kinetics of light-responses in Neurospora, we have explored the sensitivity and spatial resolution of this system. In doing so, we have been able to genetically program 2D-images in this organism. Thus, we can project a photograph on top of a Neurospora carrying a luciferase reporter under the control of a light responsive promoter and obtain back a bioluminescent pattern mimicking the original image. Thus, we have established a *live canvas* in which images are genetically processed and reconstituted with real-time dynamics. MN-FISB120043, FONDECYT 1131030.

Dr. Claudia Stange studied Biochemistry at the University of Chile and received her degree in 1996. She got her doctoral degree in Biological Science from the Catholic University of Chile in 2004. She started a postdoctoral training at the Faculty of Science, University of Chile, in 2005. Two years later she obtained the position of Assistant Professor and in 2014 she was promoted to Associate Professor at the Department of Biology, Faculty of Science, University of Chile. At her postdoc training she started working on carotenoid biosynthesis regulation in carrot to 1) understand the effect of light on root development and carotenoid accumulation, 2) determine the functionality and regulation of key genes in the carotenoid pathway and 3) to apply this knowledge in new biotechnological applications.
Carrot (*Daucus carota*) is one of the most important vegetable cultivated worldwide and the main source of dietary provitamin A. Contrary to other plants, almost all carrot varieties accumulate massive amounts of carotenoids in the root grown in darkness. She described the inhibitory effect of light on carotenoid synthesis and carrot storage root development and actually she is looking for master genes that regulate this processes by means of highthroughput approaches. She is also working on the production of carotenoids (acting as vitamins, antioxidants and pigments) or abiotic stress inducible transcription factors in plants of agronomic interest, such as apples and kiwi.

Dr. Manuel Rodriguez-Concepcion graduated in Biology from the Univ. Valencia and completed his doctoral thesis in 1995 under the supervision of Jose Pio Beltran at the IATA-CSIC (currently IBMCP) in Valencia (Spain). After a brief postdoctoral period in Valencia, he joined Wilhelm Gruissem's laboratory (Univ. California at Berkeley, USA) in 1996. On this stay, he developed and consolidated his interest on the molecular study of plant metabolism in general and isoprenoid biosynthesis and carotenoids in particular. In 1999 he returned to Spain and joined the lab of Albert Boronat (Univ. Barcelona). In 2001, he got a "Ramón y Cajal" award to start his independent research line on the molecular regulation of carotenogenesis in Arabidopsis. In 2006 he joined the Barcelona's Center for Research in Agricultural Genomics (CRAG) as a CSIC Staff Scientist CSIC and in 2010 he was promoted to Research Professor. In 2011 he spent a sabbatical year in the laboratory of Jay Keasling (Joint BioEnergy Institute, Berkeley, USA).
His work has enabled to complete the metabolic pathway responsible for the synthesis of plastid isoprenoids (including carotenoids), characterize its main enzymes, and reveal novel components involved in its transcriptional and post-transcriptional regulation. His current interests include the application of the generated knowledge in biotechnological approaches to increase the production of isoprenoids and carotenoids of interest (such as vitamins, antioxidants, or pigments) in plants of agronomic interest (such as tomatoes) and bacteria using diverse experimental approaches from molecular biology and genetics to synthetic and systems biology.

Jaime Martinez-Garcia graduated in Biology by the Universitat de València (Spain). Then he got his PhD in 1993 under the supervision of José Luis García-Martínez (IATA-CSIC, Valencia). In 1993 he moved to work with plant transcription factors in Cathie Martin's group (JIC, Norwich, UK) and in 1996 he was part of Peter Quail's team (PGEC, UC-Berkeley, USA) to work on phytochrome signaling in Arabidopsis. After a short stay in Salomé Prat lab (IBMB-CSIC, Barcelona) working on photoperiod-regulated potato tuberization, in 2001 he joined the *Institució Catalana de Recerca i Estudis Avançats* (ICREA) to establish his own research line on light control of plant development. Currently, he is a group leader at the Center for Research in Agricultural Genomics (CRAG, Consortium CSIC-IRTA-UAB-UB), in Barcelona. He is interested in analyzing the molecular and genetic mechanisms behind the plant responses to vegetation proximity and shade, working with the model plant *Arabidopsis thaliana*, a shade-avoider plant. These set of light responses are of great biotechnological and agricultural importance. In addition, he is carrying out comparative analyses between *Cardamine hirsuta* (a close relative of *A. thaliana* that tolerates vegetation proximity and shade), and *A. thaliana* as a way to dissect the genetic and molecular basis for differences in shade avoidance vs. shade tolerant species.

Dr. Luis Larrondo was born and raised in Santiago, Chile, where he received a Ph.D in Cellular and Molecular Biology at the P. Universidad Católica de Chile. With the support of the PEW foundation he conducted his postdoctoral work at Dartmouth Medical School (EE.UU) where he became interested in fungal functional genomics and circadian regulation. In 2009, he then went back to his home institution, in Chile, where he is now and associate professor and the director of the Millennium Nucleus for Fungal Integrative and Synthetic Biology. Currently, his lab works with different fungal systems studying the molecular mechanisms underlying biological oscillators, and assessing the impact that circadian clocks have on physiology and in host-pathogen interactions. Through optogenetics and synthetic biology-based approaches his lab is also exploring the design of new oscillatory circuits capable of starting and sustaining circadian rhythms.

Symposium 6

Advanced molecular modeling methods to study biochemical systems

Chairs: Julio Caballero and **Jans Alzate**, Universidad de Talca

Structural modeling using distance restraints derived from chemical cross-linking

Martinez, L University of Campinas, Brazil

Chemical cross-linking mass spectrometry provides information on the distance of residues which are solvent accessible. Surface residues are linked depending on their accessibility, local reactivity, and pairwise distances along the protein surface. Cross-linking information can be used, in principle, to obtain structural models of proteins or protein complexes. Here, we show the challenges and possible solutions for the use of these surface-accessible distance restraints for modeling protein tertiary structures. First, we model the structure of the enzyme SalBIII from the salinomycin biosinthetic pathway using distance restraints derived from chemical cross-linking, using the Rosetta software. We show that the model scoring functions of Rosetta are not adequate for the classification of the structures obtained. Therefore, a software was developed to compute surface-accessible distances and validate models as a function of the experimental results. The limitations of the cross-linking information and possible solutions are evidenced by this software. Finally, we propose an strategy to recognize the best models, and show that the experimental cross-linking data is important for the modeling process, despite its limitations. Finally, we propose a general workflow for structural modeling using chemical cross-linking data.

Bioinformatics for drug discovery in pathogens

Turjanski, A. Full professor, Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina

Available genomic data for pathogens has created new opportunities for target identification and drug discovery, including new species, resistant and multiresistant ones. However, this data must be cohesively integrated to be fully exploited and be easy to interrogate. I will present the work we have done in developing tools that include genomic annotation, structural prediction, metabolic pathways determination and target prioritization algorithms.

I will discuss the development of TARGET PATHOGENS, which allows genome wide based data consolidation from diverse sources at different processing stages focusing on structural analysis of proteins and the prediction of druggability for potential targets and compound desirability information. By allowing the integration and weighting of this information, this bioinformatic tool aims to facilitate the identification and prioritization of candidate drug targets for pathogens.

Protein Adsorption to Synthetic Materials and Its Effect on Protein Structure

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The rapid growth of applications of graphene oxide in industry and consumer products guarantees an increasing presence of this material in the environment and greater incidence of exposure in humans and other organisms. When in contact with biological fluids, graphene oxide, like many engineered nanomaterials, amasses a ``corona'' of adsorbed biomolecules on its surface, which determines cellular uptake, physiological distribution, route of excretion, and toxicology. Adsorption to the surface of graphene oxide is likely to
affect protein structure, for example, destabilizing secondary structure or causing partial denaturation, which, in turn, may elicit responses from immune and coagulation systems in complex organisms.
Here we leverage explicit-solvent molecular simulation to investigate how adsorption to graphene and graphene oxide alters the conformational equilibria of protein structural elements. We use enhanced sampling techniques to quantify how the free energies of
conformational transitions in small peptides and proteins change between the aqueous and adsorbed states. The effects on protein conformational equilibria are investigated with different arrangements of oxygen moiety density.

Design of a universal FRET-tag reveals cAMP nano-domains at β-adrenergic cascades

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Compartmentalized cAMP/PKA signaling is a well-established paradigm with important physiological implications. However, a detailed understanding of the properties, regulation and function of local cAMP/PKA signals is still missing, mostly because of the lack of proper detection tools. Aimed to improve the 3D characterization of local signaling compartments, we used molecular simulations techniques to develop a novel FRET sensor (named CUTie) that detects compartmentalized cAMP with unprecedented spatial resolution. When targeted to different multiprotein complexes located within few hundreds nanometers of each other in cardiomyocytes, CUTie reveals cAMP signals with distinct amplitude and kinetics. The nano-heterogeneity of the cAMP signal provides novel insight into fundamental mechanisms of cardiac contraction with potential relevance to pinpoint specific molecular targets for the treatment of cardiac disease.

Dr. Leandro Martínez is a graduate in Chemistry from the University of Campinas (2002), Brazil. There he also performed his master and doctoral studies on the molecular dynamics of nuclear hormone receptors. Afterwards he joined as a postdoctoral researcher the Group of Structural Bioinformatics of the Institute Pasteur, in Paris (2008), where he studied binding properties of an Anthrax pathogenic enzyme. Returning to Brazil, he joined the Institute of Physics of São Carlos of the University of São Paulo as an assistant professor, and currently holds an assistant professor position at the Institute of Chemistry of the University of Campinas. His primary research concerns the study of the molecular dynamics of biomolecules, and the development of computational tools for computational chemistry. He is the lead developer of the popular Packmol package for building initial configurations for molecular dynamics simulations.

Dr. Adrián Turjanski obtained his Master degree in Chemistry from the University of Buenos Aires in Argentina in 1999, and then his PhD in Biophysics in 2003. He conducted postdoctoral studies in the area of molecular modeling from 2003 to 2005 in the Department of Physiology and Molecular Biology, School of Sciences, University of Buenos Aires. He then conducted postdoctoral work in Bioinformatics as a 2005 Pew Latin American Fellow in the National Institute of Dental and Craniofacial Research at the National Institutes of Health. Bethesda, MD,USA. In 2008 he returned to Argentina where he is in charge of the Structural Bioinformatics Lab, in the school of sciences at the University of Buenos Aires. He is part of the research staff of the National Research and Technology Council (CONICET) as Independant Investigator. He has also been Full Professor of Bioinformatics at the University of Buenos Aires since 2008. In 2013 he became director of the Argentinian Bioinformatic Platform and in 2015 his the Director of the Center for Interdisciplinary Sciences. His research is focused in the developing and application of Bioinformatics tools for drug discovery, going from the genome to structural characterizations of proteins.

Dr. Jeffrey Comer received an undergraduate degree in Physics from the University of Akron (USA) in 2005. Thereafter, he began a PhD program at the University of Illinois (USA), using molecular simulation to better understand how nanotechnology could be used to sequence DNA in the lab of Aleksei Aksimentiev. He completed a PhD in Physics in 2010. He then moved to Chile, where he performed simulations of nanomaterial–biomolecule interaction in the group of Fernando D. González-Nilo, in association with Fraunhofer Chile Research, Universidad de Talca, and Universidad Andrés Bello. During a postdoctroal position in Nancy, France (2013–2014) supported by the Centre National de la Recherche Scientifique, he developed expertise in free-energy calculation and passive membrane transport of small molecules under Christophe Chipot. In 2014, he began his current position as an Assistant Professor in the Department of Anatomy and Physiology at Kansas State University (USA), associated with the Nanotechnology Innovation Center of Kansas State and Institute of Computational Comparative Medicine. The Comer Research Lab applies modeling and simulation to nanotechnology and biology, focusing on the adsorption of biomolecules to synthetic nanomaterials and transmembrane transport.

Dr. Sergio Pantano obtained his degree in Physics at the Universidad Nacional de San Luis, San Luis, Argentina working on statistical mechanics models of polyelectrolytes. Then he moved to Trieste, Italy, where he completed his Master and PhD at the sector of condensed matter of the Scuola Internazionale Superiore di Studi Avanzati (SISSA) in 2001. During that period he used Car-Parrinello and classical simulations to characterize a number of biological processes under the supervision of Paolo Carloni and Mauro Giacca. After short stays in San Luis and Trieste he was appointed as independent researcher at the Venetian Institute for Molecular Medicine (VIMM). In 2007 he became PI at the Institut Pasteur de Montevideo, Uruguay, where he lives currently. The group of Biomolecular Simulations headed by Dr. Pantano is among the very few research teams in South America devoted to the development of coarse-grained methods for the study of macromolecular systems related to cAMP signaling, among others.

Symposium 7

Involvement of innate immune receptors in physiological, pathological, and therapeutic immune response

Chair: María Inés Becker, BIOSONDA S.A., and Fundación Ciencia Tecnología para el Desarrollo (FUCITED).

Non-canonical pattern recognition through immune lectin receptors

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Pattern recognition receptors (PRRs) bind molecular patterns linked to the health status of tissues. On one hand, PRRs in healthy organs inform the immune system of homeostatic cellular turn over through recognition of apoptotic cells. On the other hand, during infection PRRs induce activation of the immune system through recognition of microbial compounds in the context of tissue damage. Immune lectin receptors refer to carbohydrate-binding proteins that can fine tune immune activation by modulating signalling by canonical PRRs such as Toll-like receptors. The C-type lectin receptors (CLRs) contain a carbohydrate-recognition domain that in most cases binds sugars by ligation to $Ca^{2+,}$ making the sugar-binding activity Ca^{2+} dependent. CLRs have been implicated in recognition of a wide range of microorganisms as well as endogenous molecules. In this presentation I will provide an overview of two important C-type lectin receptors, the mannose receptor (MR, CD206) and DC-SIGN (CD209). MR and DC-SIGN lack conventional signaling motifs and tend to gear immunity away from Th1 responses. As such MR and/or DC-SIGN engagement may be exploited by pathogens as means for immunoevasion. I will describe our work towards the development of novel polymeric substances capable of inhibiting MR. These compounds have shown therapeutic potential in ischemia reperfusion injury. I will also describe our recent findings regarding the contribution of MR and DC-SIGN to recognition of bacterial biofilms which highlight the potential role of biofilm-derived carbohydrates as immune modulators.

Signaling pathway of alarmin IL33/ST2 during mucosa inflammation

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The Interleukin-33 (IL-33)/ST2 axis has been implicated in numerous disease states, including asthma, rheumatoid arthritis and inflammatory bowel diseases and, more recently, in cancer and Alzheimer's disease. IL-33, a member of the IL1 cytokine family is mainly associated with the induction of T-helper type 2 (Th2) immune response through its receptor, ST2. ST2, encoded by the *IL1RL1* gene, is expressed as both a membrane-anchored receptor (ST2L) activated by IL33 and as a soluble receptor (sST2) with anti-inflammatory properties that increase during inflammation. During mucosa inflammation such as in ulcerative colitis (UC), sST2 is increased and regulated by pro- and anti-inflammatory stimuli; however, molecular regulation of sST2 expression remains unknown. Moreover, single-nucleotide polymorphisms (SNPs) in *IL1RL1* have been associated with gene expression regulation. We explored the role of IL-33/ST2 system in innate and adaptive immunity and discuss its impact on mucosal inflammatory disorders.

The role C-type lectins in modulating the TLR4-IDO axis in allergy

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Dendritic cells (DCs) are key regulators of adaptive immune responses against allergens, playing a paramount role in the induction and re-elicitation of Th2-allergic immune responses. We have previously shown that different C-type lectin receptors (CLRs), including mannose receptor (MR) and dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), play a major role in allergen recognition and uptake, and the events leading to allergic sensitization through modulation of indoleamine 2,3 dioxygenase (IDO) activity. Interestingly, the aryl hydrocarbon receptor (AhR), a transcription factor with an emerging role in immune modulation, has been implicated in IDO activation. Here we investigated how allergens and lectins modulate the TLR4-AhR-IDO axis in human monocyte-derived DCs and its effect on T helper cell differentiation.

We have demonstrated that LPS induces both IDO isoforms in DCs, with partial involvement of AhR. Additionally, we found that, like mannan, different airborne allergens can effectively downregulate TLR4-induced IDO1 and IDO2 expression, most likely through binding to the MR. Conversely, DC-SIGN might regulates TLR4-induced IDO activity in a carbohydrate specific manner. Furthermore, MR and DC-SIGN engagement was able to modulate cytokine production by DCs affecting T helper cell polarization. Interestingly, AhR and some components of the noncanonical nuclear factor kappaB pathway were shown to be downregulated after MR engagement, which could explain the regulatory effects of MR on IDO expression. This data reveals intrinsic immuno-modulatory properties of allergens, which can help to better understand how these molecules can modulates DC behaviour and the development of immune responses against allergens.

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C-type lectins involved in the immunomodulatory effects of mollusk hemocyanins

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The use of hemocyanins (Hcs) –the huge glycoproteins present in some mollusks- as a crucial ingredient in the development of vaccines againstcancer andas a nonspecific immunotherapeutic agent in bladder cancer, is well-known. These proteins induce a potent Th1 immune response. The hemocyanin from *Megathura crenulata* known as KLHhas usually been used for those purposes. However, biodiversity of hemocyanins has prompted interest in finding new candidates with better immunological properties. Thus, has emerged the Hcs from *Concholepas concholepas* (CCH) and *Fissurella latimarginata* (FLH), among others. The comparisons of immunostimulatory properties of these Hcs show differences, indicating that different HCs may activate diverse molecular and cellular pathwaysto promote Th1 immune responses. Nonetheless, it is not clear how these proteins trigger an innate like immune response leading to beneficial adaptive immune response. We proposed that one mechanism lays on the complex structure of these proteins, which culminates on its slow processing by antigen-presenting cells (APCs). In addition, the immunomodulatory effects of Hcs has been often attributed to their high carbohydrates content, being mannose the major sugar found in them. However, little is known about the interaction between Hcs and C-type lectin receptors, a family of membrane-bound glycoproteins which are expressed by APCs, which recognize and internalize specific carbohydrate antigens in a Ca^{2+} dependent- manner.

In this presentation I will describe our findings regarding the interaction between hemocyanins with some C-type receptors as the mannose receptor (MR), dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN**)** and Dectin-2.

Supported by FONDECYT 1151337

DR. LUISA MARTINEZ-POMARES, is an Associate Professor in the School of Life Sciences, University of Nottingham, UK. She is internationally recognized for her research on lectin receptors expressed by immune cells and, in particular the mannose receptor (MR). The main focus of her work is to unravel the contribution of lectin receptors to the modulation of immune responses. Dr. Martinez-Pomares combines expertise in molecular biology, biochemistry, cellular biology and immunochemistry to study the molecular characteristics of lectin receptors and identify ligands of endogenous and microbial origin. She also investigates the *in vivo* tissue distribution of receptors and their ligands as a mean to determine their contribution to immunity under steady state and inflammatory conditions. Dr. Martinez-Pomares has recently developed novel reagents to specifically reduce MR function both *in vitro* and *in vivo* that are being evaluated in a therapeutic context for the control of ischemia-reperfusion injury. Furthermore, Dr. Martinez-Pomares is exploiting her expertise in myeloid cells (macrophages, dendritic cells and neutrophils) to study the interaction of *P. aeruginosa* with the host. The main theme of this work is the identification of cellular and soluble parameters that underpin *P. aeruginosa* ability to colonise the immune compromised host. She is Section Editor Journal of Leukocyte Biology and Associate Editor Molecular Antigen Presenting Cell Biology Section of Frontiers in Immunology.

Dr. MARCELA HERMOSO RAMELLO, studied undergraduate Biochemist at the Universidad de Buenos Aires in Argentina. She obtained her Ph.D. in Biological Sciences from the Pontificia Universidad Católica de Chile in 1997, working on ciliary activity regulated by steroid hormones under the direction of Manuel Villalón. After a postdoctoral stay at the Universidad de Chile, Chile (1998 – 2002) and National Institutes of Environmental Health Sciences (NIEHS/NIH, USA), she joined the Faculty of Medicine, Universidad de Chile (2004 – present), where she changed his field of research to innate immune responses, being currently Associate Professor. The focus of the Dr. Hermoso´s Lab is mucosal innate immunity mechanisms in health and disease. The group pioneered the study of alarmin IL33 signaling pathway and receptor regulation by pro- and anti-inflammatory agents. Moreover, her group provided recently demonstration of the phagocytosis regulation by glucocorticoids and the genes related to this process.

Dr. FABIAN SALAZAR, studied undergraduate and MSc in Biochemistry and Biotechnology at the Universidad de Chile. After working as Research Assistant at the Fundación Ciencia y Tecnología para el Desarrollo (FUCITED, Santiago, Chile) in the purification and characterization of antimicrobial peptides from mollusk hemocytes with biotechnological applications, and also contributing to investigations on the immunomodulatory effects of mollusk hemocyanins in mammals, he was awarded with a PhD studentship from the Chilean Government (CONICYT). He obtained his PhD in Immunology from the University of Nottingham, UK, in 2016, investigating the role of C-type lectin receptors -in particular DC-SIGN and mannose receptor- in the regulation of the immune responses to airborne allergens. Dr. Salazar research interests include understanding how the immune system is regulated for developing new therapeutic strategies to fight back immune-related diseases.

Dr. MARÍA INÉS BECKER, obtained her PhD in Biological Sciences at the Universidad de Chile in 1989, working on mechanism of differentiation during preimplantation development of mammals under the direction of Dr. Luis Izquierdo. Then, she moved to BiosChile Ingeniería Genética, were she developing diagnostic reagents for human health using monoclonal antibodies. In 1992, Dr. Becker and Professor Alfredo De Ioannes founded BIOSONDA S.A., conducing research to discover natural immunomodulatory substances, focusing in the extensive marine Chilean resources. Dr. Becker is also Professor in the Faculty of Physical and Mathematical Sciences, at the Universidad de Chile, and in 2006, started the Fundación Ciencia y Tecnología para el Desarrollo (FUCITED), a non-profitable institution. Dr. Becker Lab´s has contributed to introduce the hemocyanins obtained from the mollusk *Concholepas concholepas* and *Fissurella latimarginata,* demonstrating that both proteins have a quaternary structure that is distinct from that of the traditional keyhole limpet (*Megathura crenulata*) hemocyanin, and both induce a potent Th1-dominant immune response with beneficial clinical outcomes. In order to better understand how these hemocyanins influence innate immune response, leading beneficial adaptive immune responses, Dr. Becker Lab´s studied their endocytosis through its engagement by some membrane C-type lectin receptors, demonstrating the involvement of some of them and also, that these hemocyanins are slowly processed by antigen presenting cells. Furthermore, provide the first evidence that macrophages undergo activation in response to structurally diverse hemocyanins and display a different temporal pattern of pro-inflammatory cytokine gene expression, along with protein secretion, which leads to an M1 polarized pro-inflammatory milieu.

Symposium 8

Plant Biology Brazil-Chile

Chair: Raúl Hererra, Universidad de Talca

Unveiling the Glycomic Code of plant cell walls to increase enzymatic hydrolysis efficiency for the production of 2G ethanol

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One of the most important barriers for development of 2G bioethanol technologies (the cellulosic ethanol) is the efficient use of enzymes during the process. The search for enzymes has been relatively successful, but the production costs are still too high. Researchers from everywhere have been looking for enzymes capable to hydrolyze plant cell walls, exploiting mainly the potential extant in microorganism's genomes. However, one of the difficulties reside on what is known as recalcitrance to hydrolysis, which is a phenomenon related to the complexity of the cell walls that are encrypted into a Glycomic Code (Buckeridge & De Souza, 2014; Tavares & Buckeridge, 2015), Our discoveries about how the polymers interact in the cell walls of sugarcane and miscanthus, two important bioenergy crops for 2G, led to the proposition of the hypothesis that the wall might be hydrolyzed more efficiently if enzyme consortia were used, instead of enzyme cocktails, following from the outside towards the inside of the wall architectural unit. This hypothesis was corroborated by the discovery that the enzyme complexes produced by *Aspergillus niger* and *Trichoderma reesei* seem to follow the expected sequence of hydrolases production when these fungi were grown on sugarcane biomass and bagasse (Borin et al., 2015). The same has been observed when we studied an endogenous mechanism extant in sugarcane that alters its own walls (see Tavares et al., 2015 for a review about such process in plants). We found such a mechanism in sugarcane roots. During development, all sugarcane roots form aerenchyma, a complex of gas spaces whose walls are altered during their formation. In this process, we detected more than 500 cell wall related genes. Among them, 49 were hydrolases. Our studies now are being directed towards the characterization of these hydrolases along with cell wall related transcription factors. We prioritize key enzymes in the disassembly of cell walls belonging to the classes of pectinases, hemicellulases and cellulases, envisaging the transformation of plants that would be capable of pre-treating themselves for further action of enzyme cocktails. We also focus on the heterologous production of sugarcane hydrolases that could be added to existing enzyme cocktails as well as to help composing enzyme consortia aiming to improve the efficiency of enzymatic hydrolysis for 2G bioethanol production.

This work was financed by the National Institute of Science and Tecnology of Bioethanol (INCT-Bioetanol)(CNPq-FAPEP) and by the Biological Engineering Program (CNPq).

Efforts to understand the ripening of *Fragaria chiloensis* fruit and its softening

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Fragaria chiloensis fruit is an outstanding berry for its aroma and exotic white-pink color, nevertheless its fast softening limits commercialization. The shelf-life of fruit depends on softening rate and extent. Softening is related to cell wall disassembling and in *F. chiloensis* fruit significant changes in pectin and hemicellulose fractions are observed during fruit ripening. In complement, several cell wall degrading enzymes take part in this process, including polygalacturonase (PG), xyloglucan endotransglycosylase/hydrolase (XTH1) and expansin 2 (Exp2), among others. The expression level of *FcPG*, *FcXTH1* and *FcExp2* increase as firmness reduction is taking place; similarly their activities increase during fruit softening. The ripening control of this non-climacteric *F. chiloensis* fruit remains unclear. To test if Aux or ABA could affect the development of ripening, *F. chiloensis* fruit were treated with these hormones. A significant change in the accumulation of *PG*, *XTH1* and *Exp2* transcripts was observed: ABA induces the expression of *XTH1* and *PG*; Aux induces *XTH1* and represses *PG*. To explain this regulatory mechanism, the promoter regions of these genes were obtained by Genome walker, and the *in silico* analysis reveals putative *cis* regulatory elements responding to hormones which could explain their differential responsiveness. These evidences had provided useful information to understand the hormonal regulation during softening of *F. chiloensis* fruit.

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Molecular analysis of sugarcane responses to drought stress

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The growing demand for bioethanol has fueled the expansion of sugarcane to regions with low rainfall levels. Even in the traditional cropping areas, drought stress is frequent, causing losses up to 40%. Therefore, the development of cultivars with improved productivity under water deficit is a major goal. Our group have been working to fill the gap regarding the understanding on the mechanisms used by distinct sugarcane varieties to cope with drought stress. To this end, we evaluated three sugarcane cultivars with high tolerance to drought (HT) and three with lower tolerance (LT) grown under field conditions. Several physiological aspects were assessed. We observed that although at the tillering phase (3 MAP), LT and HT plants had similar photosynthesis levels, HT plants were able to sustain higher photosynthesis during the grand growth period (7 months after planting). We used DNA chips to infer the changes in the transcriptome, to get insights on the biological processes triggered by water deficit. Genes related to photosynthesis, ABA biosynthesis and protein folding were associated to drought tolerance. On the other hand, the expression profile of miRNAs was complex, with strong dependence on genotype and experimental conditions. Finally, we overexpressed selected genes in tobacco and Arabidopsis plants and observed that some of them improved biomass accumulation and survival rates, reduced oxidative stress and damage to chlorophyll under water deficit conditions. In summary, we have found insights on the complex network sugarcane plants activate in response to drought, opening new venues to produce plants with enhanced tolerance to water deficit.

Plant Cell Wall Biosynthesis: The Role of Nucleotide Sugar Transporters.

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The plant cell wall is the largest source of biomass on earth. It is highly enriched in polysaccharides, which can be divided in cellulose, hemicellulose and pectin. These polymers are synthesized by glycosyltransferases, that use nucleotide sugars as substrates. The biosynthesis of cellulose takes place in the plasma membrane; however, the biosynthesis of hemicelluloses and pectin takes place in the Golgi apparatus, organelle where are located the enzymes involved in the biosynthesis of these polysaccharides. Most glycosyltransferases have their catalytic domain facing the lumen of the organelle; however, most nucleotide sugars are synthesized in the cytosol and the membrane becomes a barrier for the availability of the substrates needed for the biosynthesis of polysaccharides in the Golgi apparatus. This problem is overcome by membrane proteins known as Nucleotide Sugar Transporters and our lab had proposed that they play a critical role in the biosynthesis of the plant cell wall. We identified a gene family, composed by more than 50 members, and we have been analyzing their role *in vivo* using mutants. In this presentation we will analyze the effect on the cell wall of altering nucleotide sugar transporters for different substrates.
Supported by Fondecyt 1151335, FONDAP-CRG 15090007 and PB-16

Dr. Alejandra Moya-León studied biochemistry at the P. Universidad Católica de Chile in Chile. She obtained the Ph.D. in Agricultural Sciences (Plant Physiology) in 1994 at the Univeristy of Reading, England. She is currently full professor at the Universidad de Talca, Chile. Her research interests are focused on fruit ripening of climacteric and non-climacteric species, particularly the role of ethylene and other plant hormones in plant cell wall disassembling related to softening, as well as the production of aroma. Currently she is studying the role of transcription factors in the control of fruit softening.

Dr. Ariel Orellana obtained his Ph.D. in Biological Sciences from the P. Universidad Católica de Chile. Then, He spent three years as a postdoc in Biochemistry and Molecular Biology at University of Massachussetts Medical Center, Worcester, MA. In 1994 He was appointed as Assistant Professor at the Department of Biology, Faculty of Sciences, Universidad de Chile, Santiago. where He became Associate Professor in year 2000. In year 2004 he moved to Universidad Andrés Bello where He was appointed Full Professor and founded the Center for Plant Biotechnology, which he directed until 2014. In 2009 He was appointed as General Director for Research at UNAB, position that He left in 2010.In 2014 He was appointed as Vicerrector de Investigación y Doctorados, position that will hold until 2018. Dr Orellana has received a number of Awards and Honors during his career: PEW charitable trust fellowship and a Fogarty fellowship (1991) among many others. In 2011 He was appointed by the President of the Nation as member of the Higher Council for Science, becoming its President during 2013. He served on this position until 2014.
Dr. Orellana has published more than 60 articles in peer review journals including prestigious journals such as Science, Nature Genetics and the Proceedings of the National Academy of Science, conducting important research in Plant Genomics, ER-stress and nucleotide sugar transporters in plants.

Dr. Raúl Herrera obtained his Ph.D. in Science at The University of Reading, England and his biochemistry studies were done at Universidad de Concepción, Chile. He is currently full professor at the Universidad de Talca, Chile. His main research interests are focused on plant molecular biology and biotechnology, particularly understanding plant cell wall dynamics. He is Editor member of Journal Plant Growth Regulation (Springer) and Journal of Biochemistry Education (PABMB, ISSN:2318-8790).

Dr. Marcos Buckeridge is Associate Professor at the Department of Botany of the University of São Paulo and Director of the National Institute of Science and Technology of Bioethanol (INCT do Bioetanol), that gathers 32 laboratories in 6 states of Brazil with several collaborations in the US and Europe. From 2009 to 2012 he was also Scientific Director of the Brazilian Bioethanol Science and Technology Laboratory (CTBE), in Campinas. He is Communicating Editor *Bioenergy Research* (Springer) and Reviews Editor for *Trees: structure and function* (Springer). In 2010, Buckeridge was appointed a Lead Authors for the next Intergovernmental Panel of Climatic Changes (IPCC) report (AR5) to be released in 2014. He is the actual President of the Academy of Sciences of the State of São Paulo.

Buckeridge develops research on *Plant Cell Wall Architecture, Degradation and Bioinformatics* and on the *Physiological and Biochemical Responses of Plants to the Environment*. His research led to discoveries of mechanisms of responses of sugarcane, sorghum and several tropical species to elevated CO_2 and drought. The work on cell walls includes deciphering some mechanisms of degradation and hormonal control of storage cell wall mobilisation of xyloglucans, galactomannans, mixed-linkage-glucan and arabinogalactan. Recently the sugarcane and miscanthus cell wall structures have been described, with possible impacts on the comprehension of cell wall architecture of grasses. The model systems used for cell wall research are mainly tropical seeds, sugarcane and duckweed. Buckeridge´s work has generated 3 edited books and more than 120 publications in plant physiology, biochemistry and molecular biology of plant growth and development and cell wall metabolism.

Dr. Marcelo Menossi has a bachelor degree in Biology, University of Campinas, Brazil (1989) and a Ph.D. in Genetics, Universitat de Barcelona, Spain (1994). In 1999 he got a position as professor at the University of Campinas. In 2005 he became Assistant Professor and since 2011 he is Full Professor. He was Director of Intellectual Property and Technology Transfer at the Inova Unicamp Innovation Agency (2007-2010). His long-term interest is plant responses to abiotic stress and plant development. In maize he has uncover gene regulatory networks underlying aluminum tolerance. In the last years he has been focused in the biofuel crop sugarcane. His group worked in the production of the first DNA arrays platforms to evaluate the sugarcane transcriptome. More recently, he have been using high throughput technologies and transgenic plants to identify sugarcane genes related to drought tolerance and high sucrose content. Besides the scientific papers, his work has allowed the filing of several patents.

Oral Sessions 1
Protein Structure and Function

Structure and function of Allophycocyanin.

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Allophycocyanin (APC) is a phycobiliprotein and the main component of the core of phycobilisomes(PBS), auxiliary light harvesting protein complexes present in Cyanobacteria and red alga. APC in the core of PBS of *Gracilaria chilensis* is organized as trimers of heterodimers with different composition of subunits, each one containing a phycocyanobilin covalently bound to Cysteines: (a_3b_3), $(a^Ba_2b_3)$, $(PBa_2b^{18}b_2)$, (a_3b_3)Lc, and $(PBa_2b^{18}b_2)$Lc , some of them containing linker proteins such as the linker core(Lc) and the linker core membrane(Lcm) that contains a PB domain which can replace an alfa subunit in a trimer and that also contains a phycocyanobilin and it has been proposed as a final donor of energy to Photosystems. To provide a model for the CORE, Allophycocyanin was purified as (a_3b_3) and crystallized. The structure was solved at 2.1A resolution. The sequences of all the different components of the core were obtained and they were used to model the different compositions of trimers and a model of the core is proposed. Evidences from spectroscopy and crosslinking experiments in cyanobacterial PBS support the location of the trimers in the core. A study of the energy transfer pathways is also shown. Financial support: CONICYT Nº21120260 to JD-L and FONDECYT Nº1130256.

Characterization of Thy-1 and $\alpha_v\beta_3$ integrin interactions in crude extracts at the single molecule level using Optical Tweezers

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The neuronal surface glycoprotein Thy-1 mediates bidirectional neuron-to-astrocyte communication through its binding to astrocytic αvβ3 integrin; however, the biochemical binding parameters that characterize this interaction remained to be defined. So far, accurate measurements of binding parameters were not possible because the Fc-tagged αvβ3 integrin fusion protein was only available in the unpurified state in culture supernatants of transfected HEK293 cells. We used Optical Tweezers to quantify dissociation forces and the Dudko-Hummer-Szabo equation to determine thermodynamic and kinetic parameters of the αvβ3/Thy-1binding process. To this end, laser beam-trapped bead coated with αvβ3-Fc were allowed to contact another bead covered with Thy-1-Fc for 1 s. Thereafter, beads were separated at a constant force-loading rate, until rupture of the interaction was observed. The associated measurements permitted generating the rupture force histograms. We developed a mathematical strategy to filter rupture forces resulting from non-specific interactions with Thy-1, using the supernatant of cells transfected with mock plasmid. These interactions were corroborated studying rupture forces between Thy-1 and supernatants depleted of αvβ3-Fc, as well as between Thy-1 mutated in the integrin-binding site and the supernatant containing αvβ3-Fc. We calculated a rate at zero force k_{off}^{0} =1.3e^{-2} s^{-1}, distance $\Delta x^{\ddagger}$=0.31 nm and free energy of the transition state $\Delta G^{\ddagger}$=7.5 $k_B T$ for avb3-Fc/Thy-1 binding. Therefore, single-molecule force spectroscopy can be used to characterize bimolecular interactions important for astrocyte-neuron communication even when proteins are not available in the purified state.

Energy transfer pathways in Phycobilsome from *Gracilaria chilensis*, a time resolved fluorescence study

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Phycobilisomes (PBS) are accessory light-harvesting protein complexes found primarily in cyanobacteria and red algae. They absorb light in a wavelength range where chlorophyll is poorly efficient (the “green gap”), and transfer the energy unidirectionally to the photosystem II. This is possible thanks to chromophores phycobilins, open-chain tetrapyrroles, covalently attached to phycobiliproteins (PBPs) in specific cysteine residues. PBPs share a general architecture where they are organized as αβ heterodimers, which assemble into $(\alpha\beta)_3$trimers or $(\alpha\beta)_6$hexamers. These complexes associate and form higher-order structures, specifically the PBS core and the rods surrounding it. Efficient energy transfer is achieved through a combination of the position and geometry of the chromophores, the protein environment in which they are placed, and their spectroscopic characteristics.

Time resolved fluorescence was used to measure lifetime and anisotropy correlation to determine the rate of energy transfer between the different components of the Phycobilisome. In parallel, the rate of energy transfer between different components of the Phycobilisome were calculated using the FRET approach using chromophores position in the crystallographic structures resolved in our laboratory and the model of Phycobilisome built using the electron microscopy results.

The theoretical and experimentally values are in agree and it is possible propose a general scheme of the energy transfer in the Phycobilisome of *Gracilaria chilensis*.

Biophysical and evolutionary aspects of structural metamorphism in FoxP and RfaH transcription factors

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Evolution has selected protein sequences that spontaneously fold into a single three-dimensional structure suited for each protein's dedicated biological task. However, an increasing number of proteins challenge the "one sequence – one structure" paradigm, being able to adopt multiple dissimilar, but thermodynamically favorable structures while simultaneously switching their function, thus being termed metamorphic proteins. Among the increasing diversity of these atypical proteins, transcription factors stand out due to the key role that these structural transitions play in regulation of gene transcription. However, the molecular mechanism of these transitions and how they emerged during protein evolution remains unresolved. Here, we present our ongoing experimental and computational work on two sets of metamorphic proteins: the P subfamily of human forkhead box transcription factors, which oligomerize via domain swapping; and the bacterial virulence factor RfaH, which undergoes an unprecedented α-to-β structural rearrangement of a whole domain. Biophysical experiments aimed to address the folding-upon-binding mechanism of FoxP1 shows the presence of a monomeric intermediate preceding protein unfolding, in strong contrast to other domain swapping proteins. For RfaH, folding of the CTD into the β-fold follows a three-state folding mechanism in spite of its small size. Computational simulations based on the energy landscape theory of protein folding also show the presence of such intermediates during these structural transitions. Evolutionary analysis suggests that these transitions are a consequence of localized changes in residue identities rather than large sequence variations. Altogether, our results shed light onto the biophysical and evolutionary aspects of these protein oddities.

FLUORESCENCE-BASED APPROACH TO DETERMINE THE ROLE OF LIPIDS IN MEMBRANE PROTEIN FOLDING

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Membrane proteins (MPs) are critical for proper function of multiple cellular processes and the target of almost 60% of the pharmaceutical drugs. Despite their biological and pharmaceutical significance, little is known about their structure and how membrane lipids regulate their function and conformational changes. Here, we use a set of fluorescence-based assays and large unilamellar vesicles of controlled lipid composition to study the role of lipids in the membrane interactions of model MPs. We systematically explore how lipid headgroups regulate membrane interactions of the anti-apoptotic regulator Bcl-xL, a non-constitutive membrane protein that spontaneously insert into biological membranes in response to a signal. We found that negatively-charged lipids and lipids that induce membrane curvature stress modulate the membrane interactions and refolding of the model protein. Although experimentally more challenging, we propose that a similar approach can be applied to study structural and functional rearrangements of the glucose transporter GLUT1, a constitutive membrane protein.

Cold-adapted sugar kinase from archaea: biophysical and evolutionary study of its flexibility.

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Most extremophiles microorganisms adapted to live at low temperatures are archaea. The general mechanism adopted by psychrophilic enzymes to perform catalysis at low temperature is by reducing the free energy of the transition state rather than the Michaelis constant, K_m. This was achieved by relaxing their structures through structural modifications, among which the absence of ionic stabilizing interactions highlights. A correlation between increased structural flexibility and decreased affinity for its substrates has been shown for most of the psychrophilic enzymes studied, phenomenon that is compensated by an increase in the catalytic rate, k_{cat}. Few psychrophilic enzymes have been reported to optimize their catalytic efficiency (k_{cat}/K_m) by decreasing K_m values. Here we describe the psychrophilic enzyme from *M burtonii* (*Mb*PFK), which uses the latter mechanism as a strategy. This ADP-dependent kinase has along its structure different areas of higher flexibility compared with its thermostable homologue from *M. maripaludis (Mm*PFK). By mass spectrometry experiments we determine that segments flanking the metal-nucleotide binding motif in the psychrophilic enzyme have a higher rate of H/D exchange due to the absence of two ion pairs present in *Mm*PFK. Through ancestral sequence reconstruction of all common ancestral enzymes between psychrophilic and mesophilic enzymes we determine that the absence of these ionic interactions in the psychrophilic enzyme is an ancestral trait in the order *Methanosarcinales*. Also, when these modifications were added to the psychrophilic enzyme (*in silico* and experimental mutations), the enzyme adopts kinetic and dynamic properties similar to its mesophilic homologue.

Oral Sessions 2
Gene Expression

Physical and functional interaction between Hmo1 and the ATP-dependent chromatin remodeling complex SWI/SNF

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Chromatin dynamics has a deep impact in the different processes requiring DNA-protein interactions, such as transcription. ATP-dependent chromatin remodeling complexes are key factors in chromatin remodeling. The founding member of this family of remodeling machines is the SWI/SNF complex. Among other proteins playing a role in chromatin dynamics are High Mobility Group proteins. It has been observed that some of these proteins are able to stimulate ATP-dependent remodeling activity. We have previously determined that the yeast HMG proteins Nhp6 and Hmo1 stimulate the remodeling activity of SWI/SNF. According to our studies, Hmo1 influences more biochemical properties of this complex than Nhp6. To get further insight in the molecular mechanisms involved in the Hmo1-SWI/SNF relationship, we used different *in vitro* and *in vivo* approaches. GST pull-down assays show physical interaction of SWI/SNF with Hmo1, but not with Nhp6. We have determined that Hmo1 stimulates SWI/SNF binding to nucleosomes with the use of EMSA assays; comparative analyses testing binding to DNA and nucleosomes will also be performed, using wild-type Hmo1 and a deletion mutant lacking its C-terminal tail. ChIP-qPCR analyses suggest that Hmo1 is required for SWI/SNF binding to promoters of a fraction of its target genes, which correlates with the requirement of this HMG protein for transcriptional activity of these genes. In addition, ChIP-chip analyses comparing a wild-type strain with a *hmo1* deletion mutant, suggest that Hmo1 is also required for SWI/SNF binding to gene body regions in those genes where this complex appears to be involved in transcription elongation.

Insights into glucose sensing and its role in circadian clocks mechanisms in Neurospora.

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Circadian clocks are autonomous timers composed of interconnected transcriptional/transcriptional feedback loops. They are thought to confer a selective advantage by enabling processes to occur at appropriate times of the day. For this, environmental cues such as light and temperature shifts are critical in allowing circadian clocks to synchronize with the environment. Importantly, the clock needs to be robust to perturbations and, therefore, it is temperature and metabolic compensated. In the model organism *Neurospora crassa*, between 20-40 % of its genes are under circadian control and interestingly; many of them –such as conidiation- are under metabolic regulation and therefore, are highly sensitive to glucose levels. There is scarce information of how clock metabolic compensation is regulated in Neurospora, and on how it runs with the same period under different sugar concentrations. Through transcriptional reporters and gene expression analyses, we have characterized relevant factors involved in glucose sensing analyzing their impact in central clock mechanisms and the main output of the clock: conidiation. Thus, our data provide new insights into how glucose availability influences circadian regulation and Neurospora physiology.

Participates in the early processing of cytosolic histone H3

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The processing of cytosolic histone H3 includes the translation of its mRNA and the maturation of the protein through a “cascade of maturation”. This cascade comprises different protein complexes composed of histones, chaperones and enzymes. In this cascade, the histones H3 and H4 acquire their correct folding and the few posttranslational modifications (PTMs) detected prior to their nuclear translocation. Our previous work indicated that SetDB1 establishes the mark H3K9me1 during the histone synthesis. This observation prompted us to investigate how other enzymatic activities related to the establishment of this mark could be modulating the early processing of newly synthesized histones. By Western-blot, we found that JMJD1B, an H3K9me1/2 demethylase, is present in the cytosol of HeLa cells. Surprisingly, upon JMJD1B *Knock-down* with siRNA, H3 accumulates in the cytosol as well as histone H4, both with low levels of PTMs. NASP, a histone chaperone, also accumulates in the cytosol under this conditions. Our data suggests that JMJD1B is modulating the degradation of defective H3 and H4 in a NASP-dependent manner.

A synthetic blue-light switch to control gene expression in yeast.

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Synthetic biology has allowed the development of different optogenetic systems, which promote gene expression upon light activation. These optogenetic light switches have become a powerful tool for gene expression, heterologous proteins production and ultimately, the generation of new yeast strains with highly relevant industrial phenotypes. In this work, we implemented an optogenetic switch based on two blue light photoreceptors. The system provides inductions of over 4000-fold, as measured through luciferase reporters, revealing a highly dynamic temporal resolution. Thus, we used the optogenetic system to control the expression of genes involved in yeast flocculation, showing a strong flocculation phenotype upon light stimulation. Moreover, the reverse circuit was also assembled, showing strong flocculation in the darkness. Finally, we used the optogenetic system for heterologous expression of the biotechnologically relevant enzyme limonene synthase. Western blot analysis showed over a 100-fold of protein expression upon light activation, representing 2.5 times more expression than chemical galactose activation. Overall, the results confirmed the potential of this optogenetic tool for fine-tuning of gene expression, flocculation phenotype modulation and heterologous protein expression, which finally resulted in the development of new yeast strains with industrial applications.

Expression analysis of anti- and pro-apoptotic genes in Chilean red sea urchin (*Loxechinus albus*) coelomocytes in response to hypoxia

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Introduction: Eutrophication deteriorates many coastal ecosystems around the world, whose most important consequence is hypoxia. It has severe consequences for marine organisms, including rapid adaptive changes in metabolic organization and death. Although some aquatic animals are challenging to severe environmental hypoxia, others such as sea urchins have a restricted ability to adapt to this stressful condition. Thus, the aim of this research is to study the differential expression of genes involved in apoptosis in the red sea urchin under hypoxia. **Methods:** Cultured coelomocytes obtained from juvenile red sea urchin were exposed to normoxia (O_2 =7,4 mg/L) and hypoxia (O_2 < 2 mg/L) for 3, 6 and 9 hours. In addition, sea urchin immune cells were treated with CoCl2, a Hif-1α stabilizer. After stimulation, Cell viability was assayed using LIVE/DEAD® Viability/Cytotoxicity Kit as well as DNA laddering and caspase activity. Pro and anti-apoptotic gene expression were assayed by RT-qPCR. **Results and conclusions:** We observed time-dependent decreases in relative cell viability after hypoxia treatments. Similar observation were recorded in coelomocytes treated with CoCl2 10 mM, suggesting the participation of Hif-1α in apoptosis modulation. In addition we observed an increases in caspase laddering pattern in coelomocytes treated with CoCl2. Our results indicate that hypoxia is a powerful inducer of cell death in sea urchin immune cells. This study contributes to the comprehensive understanding of the influence of stressful conditions on the molecular mechanisms that control survival in a marine Echinoderm.

Oral Sessions 3
Biomedicine

Using of metal nanoparticles functionalized for detection of biomarkers in Gastric Cancer.

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Introduction: Gastric cancer (GC) is the third cause of death by cancer in the world and its rapid progression and lack of specific symptoms make it necessary to find new methods of detection and prevention. Gold nanoparticles have been of great scientific interest because of the many applications, especially in the area of human diseases, for its optoelectronic properties that make them useful for the development of biosensors. We have a new biomarker, Reprimo, which is inactivated at early stages of GC by methylation of promoter region. Unfortunately, current detection techniques have low sensitivity, and are useless for the diagnosis. We therefore propose the use of metal nanoparticles functionalized with oligonucleotides as a tool for early diagnostic of GC. Methodology: Gold nanoparticles of different sizes were synthesized by reduction method with citrate. They were functionalized with α-HS-PEG-ω-COOH and amino-oligonucleotides. All were characterized by UV-Vis, DLS, zeta potential, IR spectroscopy and TEM. Hybridization assays incubating various mixtures of nanoparticles with Reprimo DNA were performed and characterized by UV-Vis spectroscopy and TEM. Results: All nanoparticles maintain their optoelectronic properties after modification. The results show changes in the zeta potential as the surface oligonucleotide molecules were added. TEM analysis reveals an increase of size, attributable to the functionalization, corroborating those obtained by zeta potential. Hybridization assays demonstrate the formation of a nano-DNA hybrid by increasing the absorption intensity of the plasmonic band due to a coupling of nanoparticles plasmon. Conclusions: Our results demonstrate the potential of these systems as diagnostic tools in GC.

Enhancing the cellular uptake of chlorotoxin

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Introduction: The delivery of therapeutics into cell has been difficult due to the cellular membrane. Cell-penetrating peptides are short membrane-permeating amino acid sequences that can potentially serve as vehicle to deliver drugs into cells. However, they lack specificity. Chlorotoxin (CTX), a disulfide-rich peptide isolated from the venom of the scorpion *Leiurus quinquestriatus,* has several promising biopharmaceutical properties, including the ability to bind preferentially to cancer cells. Recently, CTX has also been shown to penetrate cells, suggesting that CTX can be used for intracellular delivery of therapeutic compounds specifically to cancer cells. ***Results:***Here, we have studied the structural features of CTX that affect its cell-penetrating properties. By increasing the number of arginine and tryptophan residues, we showed that arginine residues facilitate the cellular uptake of CTX. Particularly, a double mutant CTX[K15R/K23R] showed a two-fold improvement in uptake compared to CTX. We also showed that mutant CTX[K15R/K23R/Y29W] increases the internalization. We have also demonstrated that the fluorescent dye attached to CTX influences its cellular uptake with Cy5.5™ having a larger effect compared with Alexa Fluor® 488. ***Conclusion:*** In this study, we have showed that conservative amino acid substitutions can improve the potential of CTX as a vehicle for delivery of molecules into cells.

The Down syndrome critical region gene 1, RCAN1, regulates mitochondrial dynamics and metabolism in cardiomyocytes and Down syndrome induced pluripotent stem cells

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Introduction. Altered calcium handling and mitochondrial function are hallmarks of heart disease and both influence heart susceptibility to ischemia reperfusion (I/R). The Down syndrome (DS) critical region gene 1, DSCR1 or RCAN1 is a feedback inhibitor of calcineurin, a calcium-activated phosphatase involved in cardiac remodeling. **Methodology.** We used advanced imaging techniques to dissect RCAN1\'s role in mitochondrial dynamics and function. SiRNA was used to deplete RCAN1 from neonatal rat ventricular myocytes (NRVMs) and DS induced pluripotent stem cells (iPSCs). **Results.** In NRVMs, confocal imaging showed an increase in the number of mitochondria per cell and a decreased individual mitochondrial size, indicative of increased fission in the absence of RCAN1. There was also a corresponding decrease in the phosphorylation of the fission protein DRP1, a calcineurin substrate. Consistent with increased fission, the siRCAN1-depleted NRVMs showed decreases in ATP production, mitochondrial membrane potential, oxygen consumption, and mitochondrial calcium uptake. We also used DS iPSCs, where it is has been described the presence of increased RCAN1 levels. DS iPSCs presented a decreased number of mitochondrial per cell together with an increase in the mitochondrial individual mean volume, consistent with mitochondrial fusion. Additionally, DS iPSCs presented high levels of basal oxygen consumption and proton leak-induced respiration, indicating high levels of uncoupling related to ROS production. Increased mitochondrial fusion and respiration in DS iPSCs were rescued with the RCAN1-siRNA. **Conclusion.** These data suggest that RCAN1 preserves mitochondrial fusion by inhibiting calcineurin-dependent activation of DRP1, thus preserving mitochondrial network connectivity.

Maternal age effect and severe germ-line bottleneck in the inheritance of human mitochondrial DNA

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The manifestation of mitochondrial DNA (mtDNA) diseases depends on the frequency of heteroplasmy (the presence of several alleles in an individual), yet its transmission across generations cannot be readily predicted owing to a lack of data on the size of the mtDNA bottleneck during oogenesis. For deleterious heteroplasmies, a severe bottleneck may abruptly transform a benign (low) frequency in a mother into a disease-causing (high) frequency in her child. Here we present a high-resolution study of heteroplasmy transmission conducted on blood and buccal mtDNA of 39 healthy mother–child pairs of European ancestry (156 samples in total, sequenced at ~20,000× per site). On average, each individual carried one heteroplasmy, and one in eight individuals carried a disease-associated heteroplasmy, with minor allele frequency ≥1%. We observed frequent drastic heteroplasmy frequency shifts between generations and estimated the effective size of the germ-line mtDNA bottleneck at only ~30–35 (interquartile range from 9 to 141). Accounting for heteroplasmies, we estimated the mtDNA germ-line mutation rate at 1.3 × 10−8 (interquartile range from 4.2 × 10−9 to 4.1 × 10−8) mutations per site per year, an order of magnitude higher than for nuclear DNA. Notably, we found a positive association between the number of heteroplasmies in a child and maternal age at fertilization, likely attributable to oocyte aging. This study also took advantage of droplet digital PCR (ddPCR) to validate heteroplasmies and confirm a de novo mutation. Our results can be used to predict the transmission of disease-causing mtDNA variants and illuminate evolutionary dynamics of the mitochondrial genome.

Death induced by inhibition of CK2 in colon cancer cells: Vacuolization or autophagy?

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Colon cancer is linked to deregulation of several kinases, including CK2, a highly conserved and active serine/threonine kinase that regulates a large number of cell processes. In fact, CK2 inhibition and silencing decrease viability and migration, as well as increase apoptosis in several cancers, including colon. Pharmacological inhibition of CK2 using CX-4945 induces autophagy in glioblastoma and lung cancer cell lines, with no current evidence in colon cancer. Our results show that CX-4945 induces a rapid and efficient cell death in human DLD-1 colon cancer cells, but they exhibit large vacuoles that resemble autophagy. For in vitro study of autophagy, protein levels of LC3II after CK2-overexpression or using CK2-inhibitor CX-4945 were evaluated. In vivo analysis was performed using different markers of endosomes and lysosomes. Our results indicate that vacuoles formed under CX-4945 treatment are labeled with the acidic marker lysotracker. Moreover, the membrane is marked with the protein Rab7. Additionally, it was determined that membrane vacuoles are CD63 and LAMP1 positives, both markers of late endosomes and lysosomes. Likewise, by using an in vivo endocytosis assay, we demonstrated that vacuoles have internalized dextran. Finally, by overexpressing GFP-LC3, we show that some vacuolar subpopulation had LC3 in its membrane. Taken together, our results suggest that CX-4945 in colon cancer cells generates vacuoles (late endosomes) which, at a later stage, fuse with lysosomes, leading to lysosomal cell death. However, whether this mechanism is linked to autophagy is still unknown.

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Decreased lipogenic markers in prevention of hepatic steatosis by dietary Rosehip oil supplementation in high fat diet-fed mice.

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Introduction: Liver steatosis is a physiopathological alteration characterized by an excessive intake of saturated fats and/or carbohydrates and depletion of omega-3 fatty acids, and is developed as a result of the imbalance between the acquisition/de novo synthesis of fatty acids and β-oxidation or VLDL export of them. Rosehip oil (RM, Rosa rubiginosa) has a high content of alpha-linolenic acid (ALA), oleic acid and antioxidants. Our group has shown that RM administration decreases hepatic steatosis induced by a high fat diet (HFD). Objective: To evaluate changes in lipogenic markers associated to prevention of hepatic steatosis by RM supplementation in mice. Methodology: Male C57BL/6J (n = 9 per group) were fed for 12 weeks and divided into the following groups: (i) control diet (10% fat, 20% protein, 70% carbohydrates); (ii) control diet plus RM (1.94 mg ALA /g body weight/day, American Bioprocess Ltda); (iii) HFD (60% fat, 20% protein, 20% carbohydrates); (iv) HFD plus RM. Parameters of liver steatosis (histology, triglyceride content), perilipin-2 and -5 levels [qPCR, indirect immunofluorescence (IFI)] and nuclear SREBP-1c levels [immunohistochemistry (IHC)] were evaluated. Results: It is observed a significantly increase (One-way ANOVA, Newman-Keuls test, $P < 0.05$) in perilipin-2 levels and nuclear SREBP-1c localization in HFD group when compared to control groups. However, a strong decreasing of these markers is observed in HFD plus RM group with respect to HFD only. No differences in perilipin-5 levels were observed. Conclusion: The reduction of perilipin-2 levels and SREBP-1c activation may contribute to prevent hepatic steatosis by RM administration.

Oral Sessions 4
Immunology

Chemokines expressed in colorectal cancer are important in tumor-associated macrophages (TAMs) profile.

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Introduction: Chemokines are small secreted proteins that participate in the recruitment of hematopoietic cells during the immune response. Furthermore, chemokines have been related with the rolling and holding of tumor-associated macrophages (TAMs), promoting progression and metastasis of cancer, such as prostate, breast, lung and colon. The aim of this study was to investigate chemokines that may participate in the recruitment of TAMs and its association with colorectal cancer progression. Methodology: Tumor and healthy mucosa were obtained from patients with colon cancer (n= 12, TNM stage=1-3) that underwent surgery. Protein extract from tissue were performed with RIPA buffer and quantified by BCA assay. The concentration of chemokines that have a role in the macrophages recruitment such as CCL2, CCL3, CCL4, CCL5 and CX3CL1, were measure by Luminex assays. Plasma level of CCL3 were evaluated by ELISA. Statistical analysis were performed using non-parametric Wilcoxon matched pair test, considering $p<0,05$. Results: Levels of CCL3 (Median= 70,83 pg/g) and CCL4 (Median= 39,44 pg/g) were higher in tumors than in healthy tissue (Median= 33,34 and 33,17 pg/g, respectively) with *p=0,0034* and *0,0161*. CCL3 levels tended to increase in early stage tumors. However, plasma levels of CCL3 were no correlate with the tumor stage. No significant differences in CCL2, CCL5 and CX3CL1 were observed among tumor and healthy tissue. Conclusions: High expression of CCL3 and CCL4 in colon cancer could induce the infiltration of TAMs and represents a potential prognostic biomarker and a therapeutic target to modulate the macrophage participation in cancer progression.

MICRORNA 378a-3P, A NEW MOLECULAR MECHANISM OF IL-33 REGULATION IN ULCERATIVE COLITIS

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Introduction: IL-33, a member of a IL-1 family, is produced mainly by epithelial cells and its binding to ST2L receptor induces secretion of pro-inflammatory cytokines by immune cells. Intestinal mucosa of active ulcerative colitis (aUC) patients presents increased levels of IL-33. MicroRNAs are deregulated in this disease and in particular the miR-378a-3p is a potential candidate to control IL-33-mRNA expression. Aim: to study the association of IL-33-mRNA levels with the miR-378a-3p expression. Methodology: microRNAs and mRNAs differentially expressed, according to inflamed vs. non-inflamed intestinal mucosa by microarray analysis (from 8 UC patients), were selected. MicroRNAs and mRNAs with a high fold-change (FC) in microarrays were detected by TaqMan and RT-PCR, respectively, in 20 UC patients, 13 inactive (iUC) patients, and 12 controls. Furthermore colonic epithelial cells (HT-29) were exposed to inflammatory stimuli (TNFα) to evaluate selected microRNAs and mRNAs expression. Results: Inflamed mucosa was associated with increased expression of 26-microRNAs+432-mRNAs, and decreased expression of 22-microRNAs+314-mRNAs. IL-33-mRNA was increased (3.7 FC) and miR-378a-3p was decreased (-3.4 FC), and concomitantly had a target sequence in the IL-33-mRNA 3'UTR with a high interaction score (TargetScan: 8mer). aUC patients showed decreased miR-378a-3p and increased IL-33-mRNA levels, compared to iUC and controls (Mann-Whitney test), and both molecules were inversely correlated (*Pearson-r*=-0.35, *P*=0.04). MiR-378a-3p and IL-33-mRNA were also inversely correlated (*Pearson-r*=-0.49, *P*=0.0093) in HT-29 cell assays. Conclusions: These results suggest a new mechanism of regulation of alarmin IL-33 by miR-378a-3p, a metabolism-controlled miRNA.

Adipose cell NLRP3 inflammasome upregulation through calcium sensing receptor activation

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Adipose tissue (AT) expansion can be accompanied by a proinflammatory status, resulting in local metabolic dysfunction and inflammation, with consequences at a whole body level. We have described that activation of the G protein-coupled calcium sensing receptor (CaSR) induces a proinflammatory state in adipose cells, linking it with AT dysfunction. On the other hand, the NOD-like receptor protein 3 (NLRP3) inflammasome is a signaling platform assembled in response to danger signals, leading to catalytic cleavage of caspase-1 (CASP1) that controls maturation and secretion of the proinflammatory cytokine interleukin (IL)1β. Since NLRP3 inflammasome activation has been associated to AT inflammation and dysfunction, we studied if CaSR activation stimulates the expression of NLRP3 components in human adipose cells. LS14 preadipocytes or adipocytes were treated overnight with 2μM cinacalcet (a CaSR activator). For CaSR silencing, preadipocytes were transfected with siRNA targeting CaSR or a scrambled sequence (control). Cinacalcet stimulation enhanced mRNA expression of the inflammasome components NLRP3 (65%, P=0.004), apoptosis-associated speck-like protein containing a CARD (ASC, 33%, P=0.008), CASP1 (159%, P=0.0004) and IL1β (62%, P<0.0001). CaSR silencing prevented this increase. A positive correlation (Sperman) was observed between the responses of ASC and IL1β (r=0.42, P=0.045). In LS14 adipocytes cinacalcet increased IL1β expression by 70% (P=0.015) but had no effect on the expression of the other NLRP3 components. These data are the first report of CaSR-dependent inflammasome activation in preadipose cells and suggests a differential pattern of gene expression response among the cells conforming the white adipose tissue.

Simvastatin decreases inflammation and fibrosis on a murine model of chronic Chagas´s cardiopathy through 15-epi-lipoxin A_4 production

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In Chronic Chagas Cardiopathy (CCC), the infection with the protozoon *Trypanosoma cruzi* (*T. cruzi*) activates vascular endothelium and increases the expression of proinflammatory cytokines. This proinflammatory state is mediated by activation of nuclear factor kappa B (NFκB) that is involved in the increased expression of cellular adhesion molecules (CAMs) to encourage inflammatory cell recruitment. On the other hand, statins reduce inflammation in the vascular endothelium, NFκB activation, and E-CAMs expression. It has been described that these effects are mediated by the production of 15-epi-lipoxinA_4 through the action of 5-lipoxigenase (5-LO). 15-epi-lipoxinA_4 is a pro-resolutory of inflammation eicosanoid, which inhibits NFkB activation. Therefore, we evaluated the impact of this therapeutic approach on inflammation and fibrosis of a murine model of CCC. Sv/129 WT and 5-LO-/- mice infected with 1000 blood trypomastigote of Dm28c strain were treated with benznidazole 100 mg, simvastain 40 mg, or 15-epi-lipoxin A4 25ug/kg/day for 20 days, starting at the 30th day post infection (p.i.). Animals were euthanized at day 80 p.i. to obtain blood and cardiac tissue for parasite load, inflammatory infiltrate and fibrosis analysis. As expected, benznidazole 100 mg/Kg/day decreased parasite load, inflammation and fibrosis on WT and 5-LO-/- mice as well as simvastatin 40 mg on WT mice. Nevertheless, in 5-LO-/- mice, simvastatin 40 mg did not decrease the parasite load, inflammation and fibrosis completely, but it did occur on 15-epi-lipoxinA_4 exogenous administered mice. In conclusion, Simvastatin, through 15-epi-lipoxin A_4 action, provides an anti-inflammatory environment on cardiac tissue infected with *T. cruzi*.

Interleukin 33, a potential candidate for epithelial mesenchymal transition activation in colorectal cancer.

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Introduction: Interleukin 33 (IL33), is synthesized by epithelial, endothelial cells and fibroblasts. Recently, has been acknowledged a protumorigenic and proangiogenic role attributed to IL33 in cancer. IL33 secreted from cancer associated fibroblasts(CAF), activates epithelial mesenchymal transition(EMT) in head-neck cancer and higher content was related with poor survival. However, in colorectal cancer(CRC) its role is controversial. **Aim:** to evaluate IL33 expression in CRC-CAF and its role in EMT. **Methods:** Tumoral and normal tissue from twelve CRC patients(stage 1-3) were obtained and IL33 protein was measured by ELISA. CAFs and normal fibroblasts (NF) from CRC patients with different promigratory profile were subjected to microarray analysis. Also, E-cadherin protein and migration capacity were assessed in CRC cells (HT-29, LIM1215, SW480 and HCT116), exposed to IL33 by western blot and transwell migration assay, respectively. **Results:** IL33 protein levels showed no difference between tumoral and normal tissue (Wilcoxon paired, p=0.266) but there was a tendency for increased IL33 content in higher stage (Kruskal-wallis, p=0.06). However, high migratory-CAF expressed increased IL33 transcript levels compared to low promigratory-CAF and NF. Moreover, IL33 induced migration in HCT116, LIM1215, and HT-29 cells. Additionally, IL33 down-regulated E-cadherin expression in HT-29 and HCT116 cells. SW480 cells did not show changes in migration nor E-cadherin expression. **Conclusions:** These results suggest that IL33 activates EMT favoring migration and that CAF seems to be a potential source of this cytokine highlighting its role in CRC pathogenesis. More samples will be reunited to define differences in IL33 content in tumors.

Glucocorticoids impair phagocytosis and inflammatory response on macrophages infected with a Crohn´s Disease-associated pathogenic bacteria

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Glucocorticoids (GC) are a common treatment for inflammatory disorders; however, prolonged use can predispose people to bacterial infection. Studies addressing GC effects on bactericidal and inflammatory activity of infected macrophages are controversial. Here, we examined GC-treated macrophages and their interaction with adherent-invasive *E. coli* (AIEC) strain, CD2-a, an intestinal mucosa pathogen-associated to Crohn´s that survives phagocytosis. Aim: To determine GC effects on bactericidal and inflammatory activity of macrophages infected with CD2-a. Methodology: THP-1 cells-derived macrophages were infected with CD2-a in the presence or absence of dexamethasone (Dex), and a mRNA microarray was performed. Differentially expressed mRNAs were confirmed by TaqMan-qPCR. Amikacine-protection assay was used to evaluate phagocytic and bactericidal activity of Dex-treated macrophages and infected with *E. coli* strains (CD2-a, HM605, NRG857c, HB101). Cytokine secretion and inflammatory phenotype of macrophages were evaluated by ELISA and flow cytometry, respectively. Results: Microarray analysis showed that CD2-a, Dex and CD2-a+Dex have differential inflammatory genes profiles, many with unique expression pattern in CD2-a+Dex condition. Canonical pathway analysis showed a decreased phagocytosis signaling on Dex-treated macrophages, and an anti-inflammatory polarization on CD2-a+Dex macrophages. Amikacine protection assay showed reduced phagocytosis capacity by Dex. TaqMan-qPCR confirmed Dex inhibition of three phagocytosis-associated genes. All bacteria strains induced TNF-α, IL-6, IL-23, CD40 and CD80 levels that were inhibited by Dex. Conclusions: GC-induced decreased phagocytosis and an anti-inflammatory polarization upon *E. coli* macrophage infection suggesting that AIEC infected patients, under this treatment, could have an impaired bacterial clearance.

Oral Sessions 5
Molecular cell biology and plant biology

Caveolin-1 in exosomes from metastatic breast cancer cells enhances metastasis in a novel model of intraperitoneal carcinomatosis

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Introduction

Breast cancer is the leading cause of cancer-related death in women worldwide. In this regard, Caveolin-1(CAV1), a multifunctional protein that is upregulated in final stages of cancer, promotes invasiveness of tumor cells. High levels of this protein are found in exosomes from advanced cancer patients. Exosomes, small approximately 100 nm vesicles that transport proteins and micro-RNAs, have been implicated in metastasis. Thus, an intriguing possibility is that CAV1 in exosomes may enhance the metastatic potential of tumor cells by mechanisms that remain to be defined.

Methodology

Exosomes were purified from the supernatant of metastatic MDA-MB-231 wild-type(wt), MDA-MB-231(shCAV1) (containing a “small hairpin” directed against CAV1) and MDA-MB-231(shControl) by using Exo-spin (Cell Guidance Systems). Microparticle size and distribution was determined by transmission electron microscopy and Nanosight analysis. Exosome markers and CAV1 were detected by immunoblot. The invasive potential of cancer cells stimulated with exosomes was evaluated in matrigel assays. A peritoneal carcinomatosis model was developed to evaluate the role of CAV1 by injecting immunodeficient mice with MDA-MB-231 and non-metastatic T47-D cells along with MDA-MB-231(wt), shControl and MDA-MB-231(shCAV1) exosomes via intraperitoneal inyection.

Results

The invasive potential of cancer cells increased when stimulated with MDA-MB-231(wt) and shControl exosomes. Notably, mice injected with MDA-MB-231 and T47-D cells along with MDA-MB-231(wt) and shControl exosomes displayed increased metastasis and ascites volume within the peritoneal cavity compared to mice injected with MDA-MB-231(shCAV1) exosomes for which little metastasis and essentially no ascites were detected.

Conclusions These results suggest that intercellular communication promoted by exosomes with CAV1 favors metastasis.

Adenosine A3 Receptor Elicits Chemoresistance Mediated by the Multiple Resistance-associated Protein-1 in Human Glioblastoma Stem-like Cells

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Introduction: MRP1 transporter is related with Glioblastoma Multiforme (GBM) malignancy and Multiple Drug Resistance (MDR) phenotype. It has been evidenced MRP1 is controlled by the adenosine axis trough CD73 ectonucleotidase activity and A3 adenosine receptor (A3AR) activation in GBM bulk cells. However, in Glioblastoma Stem-like Cells (GSCs), cells responsible for the tumorigenic and chemoresistance capabilities on GBM, this mechanism is unknown. The aim of this study was to identify the effects of adenosine on the MDR phenotype in GSCs and its implications as a chemosensitizing agent. M&M: Expression of MRP1, A3AR and CD73 of GSCs were evaluated (RT-qPCR, western blot and flow cytometry). HPLC was utilised to determine adenosine concentration. Cell viability/proliferation and MRP1 activity were evaluated using selective antagonists of CD73 and A3AR. In addition, the signalling pathway involve in MRP1 expression was evaluated. Finally, we evaluated the chemosensitizing effect of an A3AR antagonist on an in vivo xenograft model. Results: GSCs have increased intrinsic capacity to generate extracellular adenosine, thereby controlling MRP1 expression/activity via A3AR activation. We evidenced PI3K/Akt and MEK/ERK1/2 signalling pathways were implicated in the expresion of MRP1. In vitro blockage of A3AR had a chemosensitizing effect, enhancing antitumour drug actions and decreasing cell viability/proliferation of GSCs. Antagonist of A3AR also had a chemosensitizing effect in vivo, enhancing the effectiveness of vincristine (drug substrate of MRP1), decreasing tumour size and expression levels of Stem cell markers and Ki-67 proliferation indicator. Conclusion: We have demonstrated the susceptibility of GSCs to be chemosensitized by blockage of A3AR.

Activation of Angiotensin II type 1 receptor induces autophagy in vascular smooth muscle cells

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Vascular smooth muscle cells (VSMCs) are an essential blood vessel component involved in vascular tone regulation. During hypertension, Angiotensin II (Ang II) levels are increased inducing vascular remodeling and cardiovascular diseases. Here we evaluate the effect of Angiotensin II type 1 receptor (AT1R) activation by Ang II on autophagy in VSMCs. VSMC A7r5 from rat aorta were treated with Ang II (100 nM) for 24 h. Autophagy was evaluated by LC3II/GAPDH, Beclin-1, Atg12–Atg5 levels and autophagic flux in presence and absence of chloroquine. A7r5 cells treated with Ang II 100 nM for 24 h showed an increased conversion of LC3I to LC3II, evidenced by an increase of LC3II/GAPDH ratio. This effect was due to an increased autophagic flux because treatment with Ang II 100 nM and chloroquine 30 μM also showed LC3II accumulation. Two proteins involved in early steps of autophagy, Beclin-1 and Atg12–Atg5 also were increased after stimulation with Ang II 100 nM. Blockade of the AT1R by Losartan showed a reduction on Ang II-induced LC3II/GAPDH ratio. Our data suggest that Ang II induces autophagy in VSMCs due to AT1R activation. This process could play a key role in VSMC phenotype switching induced by Ang II in cardiovascular diseases.

The antisense non-coding mitochondrial RNAs as therapeutic targets in cervical and ovarian cancer

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The family of non-coding mitochondrial RNAs (ncmtRNAs) displays differential expression according to proliferative status. Normal proliferating cells express the sense (SncmtRNA) and antisense (ASncmtRNAs) transcripts, whereas tumor cells express the SncmtRNA and down-regulate the ASncmtRNAs. Knockdown of ASncmtRNA, by chemically-modified antisense oligonucleotides (ASOs), induces apoptotic cell death, without affecting viability of normal cells. Moreover, in a syngeneic murine model of B16F10 melanoma in C57BL6/J mice, injection of an ASO targeted to the mouse ASncmtRNA retarded tumor growth and metastasis. In this work, we focused on prevalent gynecological malignancies, cervical and ovarian cancers, which display high mortality in women worldwide (Globocan, 2012). Mortality is associated to tumor relapse and metastasis, which has been attributed to the presence of Cancer Stem Cells (CSCs). These cells are resistant to conventional antineoplastic drugs and have the potential to regenerate primary tumors, thereby inducing metastasis, which is largely the basis of cancer mortality. We found that knockdown of ASncmtRNAs elicits death of ovarian cancer cell lines and alters the ability to form spheres in primary culture samples from both cancer types. Also, we established a subcutaneous xenograft model from primary cultures in order to evaluate the *in vivo* efficacy of the ASO treatment. After tumors reached 100 mm^3, mice were given 10 ASO injections, where we observed a strong reduction in tumor growth rate. Our model suggests that down-regulation of the ASncmtRNAs constitutes a vulnerability of cancer cells, because this therapy can eradicate both tumor bulk cells and CSCs.

In-silico analysis of the structure and binding site features of FcEXPA2 an α-expansin protein involved in ripening of *Fragaria chiloensis* fruit

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Expansins are proteins with no catalytic activity, which have been associated to several processes during plant growth and development, as well as, fruit ripening. The canonical expansin structure consists of one domain responsible for substrate binding (D2) and another domain (D1) of unknown function, but essential for activity. Although the effects of expansins on cell walls and cellulose fibrils are known, the molecular mechanism underlying their biophysical function is poorly understood. In *Fragaria chiloensis* one expansin gene (*FcEXPA2*) was identified with high homology to other plant α-expansins. Firstly, the full-length sequence was obtained, and using qRT-PCR, transcript accumulation was determined during ripening of *F. chiloensis* fruit, and matched with softening. A 3D model was built by comparative modeling methodology, the model obtained showed a D2 with a β-sandwich structure, and a D1 with a similar structure to the catalytic core of endoglucanase from *Humicola insolens*. The protein-ligand interaction was evaluated using molecular dynamics simulations, and the results shown that FcEXPA2 can form hydrogen bonds with free cellulose chain in a twisted conformation and that the twisting is chiefly induced by means of residue Asp105 located on D1, which has been shown to be essential for expansin activity. These results suggest that FcEXPA2 could move on the surface of cellulose and disrupt hydrogen bonds by twisting glucan chains. Our results provide a dynamical view of the expansin–substrate interactions at the molecular scale and help shed light on the expansin mechanism during the ripening of the fruits.

CRISPR/Cas9 as a cleavage system for marker genes in plants.

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The CRISPR/ Cas9 system is a versatile and effective tool for genome editing that has been used in many plants species. Among the modifications that can be introduced into the genome, the most common are small INDEL, but, CRISPR/ Cas9 can also be used to produce the deletion of a specific sequence. It is this last feature, which allows us to propose the CRISPR/ Cas9 system as a potential tool for selection marker excision without using a site-specific recombination system, as other commercial techniques. For this purpose, by means of Synthetic Biology using the Gibson assembly system, we constructed a complete vector composed of the *Cas9* coding sequence under the temperature inducible *Hsp18.2* promoter and a gRNA directed to excise the selectable marker gene. As the selectable marker, we chose the *dao1* gene, which codes for a D-aminoacyl oxidase. This enzyme metabolizes D-alanine, which is toxic to plants, into a non-toxic compound, and metabolizes D-valine, which is not toxic, to a toxic molecule. Thus, the same gene will be used as a positive and negative selection marker for gene excision analysis in transgenic plants. Therefore, functional evaluation of the CRSPR/Cas9 cleavage systemwas assessed in plant before and after induction with high temperature.

Oral Sessions 6
Computational biology and Bioinformatic and Enzymes and Metabolism

Structure-based identification and preliminary pharmacological characterization of CHB013, a novel selective connexin hemichannel blocker.

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In recent years, it has been demonstrated that under physiological conditions connexin hemichannels (CHs) play relevant roles in autocrine and paracrine cell-cell signaling providing a membrane pathway for releasing extracellular signaling molecules such as ATP and NAD^+. However, in several pathological conditions the activity of connexin hemichannels is upregulated and contributes to the outcome of cellular degeneration. The currently available connexin hemichannel blockers also block gap junctions, which play relevant roles in coordinating numerous electrical and metabolic responses of cellular communities. Therefore, selective connexin hemichannel blockers might be useful to unravel the CHs role in pathology and the design rational therapeutic treatments for diverse diseases. In the present work, we report the successful use of a structure-based virtual screening approach for the identification of novel chemical entities targeting connexin hemichannels. We have identified a selective connexin hemichannel blocker (CHB013) which blocked unitary current events in hemichannels formed by connexins 26, 32, 43 and 45, but not gap junction channels formed by these connexins, pannexin 1 or other related channels. Preliminary pharmacological evaluation shows that CHB013 prevents PTZ-induced epilepsy and improves muscle strength in muscular dystrophy mice models. The lead compound CHB013 binding characteristics and physicochemical properties are used as starting point for the development of a novel generation of potent and specific connexin hemichannel modulators.

Vibrational energy propagation in protein structures: implications for allosteric communication.

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Allosteric communication is a central an unsolved problem in structural biochemistry. Despite the availability of several mapping algorithms for the tracking of allosteric pathways its construction lack a fundamental ingredient, that is, a description of the vibrational dynamics of the residues that compose and allosteric pathway. This description is essential of we want to understand the process of allosteric communication as a process that involves energy flow. In this presentation will show recent advances in energy flow along in protein structure. This research has been done with the usage of classical and quantum molecular dynamics. The main finding of this research offers an explanation for spectroscopic observation of asymmetrical energy flow in organic molecules. This phenomenon is also predicted in our research for amide groups that give rise to backbone-backbone hydrogen bonds of proteins, thus this effect it may have a relevant role in the process of energy diffusion through allosteric pathways and for the process of vibrational activation of catalytic sites in proteins. The PDZ-2 and to the TRPV1 proteins have been analysed and the results suggests that the phenomenon of allosteric communication indeed implies a vibrational energy flow along the protein structures.

Coarse-grained simulations show secondary structure content and phase separation dependence in the adsorption of the cold regulate protein COR15A of *Arabidopsis thaliana*

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Cold has a major influence on plant growth, caused by cellular dehydration generated by extracellular ice crystallization. In those states, plants start to accumulate the cold-regulated protein COR15A. COR15 proteins play a crucial role by improving cell resistance during cellular dehydration. According to experimental evidence, COR15 proteins are Intrinsically Disordered Proteins (IDP) on water, but can acquire secondary structure during cellular dehydration in order to stabilize membranes. It has been proposed that the protein COR15A (localized in chloroplast) is able to interact peripherally to membranes by its amphipathic nature on both in vitro and in vivo assays, where a proper interaction/stabilization is directly related in the dehydration state. On a previous work, we were able to obtain 3 different conformations of COR15A at different dehydrations conditions (Folded, Partially folded, Unfolded) by Molecular Dynamics simulations. On that concern, by using Coarse-grain (CG) simulations we aimed to explain how cellular dehydration affect membrane stabilization by analyzing the adsorption of the 3 conformations of COR15A in chloroplast membranes and several thylakoid-phospholipid mixture membranes.

We found that indeed was the folded conformation of the protein that adsorbs preferentially, mediated by interacting with the hydrophobic interface of the protein, followed by the partially-folded and the unfolded conformation. With respect to the mixture membranes, we found that lipid raft generation of the thylakoid lipids was crucial in order to a proper adsorption, were the proteins interact preferentially with the most polar headgroups, proving the importance of cellular dehydration in membrane stabilization.

Towards an accurate prediction of active DNA transposable elements: HMMs perform better than Repeatmasker

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Eukaryotic transposons are DNA sequences able to move inside a genome. They are characterized by a sequence that encodes a transposase protein of ~300 aminoacids and flanking it, short terminal inverted repeats of ~30bp. They are difficult to predict computationally because: 1. Due to their activity, there are many copies, or paralogous, of the transposons of a family in a genome; 2. Due to mutation, a high diversity of sequences has resulted, and as consequence; 3. Many transposons are incomplete or mutated enough to render the element inactive. In order to circumvent these issues, we generated Hidden Markov Models (HMMs) for 12 families of eukaryotic transposases. HMMs are an appropriate technique for searching evolutionary divergent sequences. During organism's development, transposons activity is regulated by piRNAs. This regulation occurs via Watson-Crick base pairing between the piRNA and the transposase transcript.

In order to test the ability of our models to predict active transposases, we used as reference the mapping of known piRNAs sequences of an organism on its own genome, and compared it to our transposase predictions, and to those made by RepeatMasker, the current gold standard software for prediction of mobile elements. We found that, while RepeatMasker has a higher absolute number of predictions, its sensitivity and selectivity as classifier of active transposases is lower than our HMMs for all tested organisms. Although, there is a lot of room for improvement, these results are a step towards the improvement of the accuracy of prediction of active transposases.

DYRK family in seminiferous epithelium and its potential role in the regulation of glycogen metabolism.

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The CMGC group of Serine/Threonine kinases includes a conserved family called DYRK (Dual-specificity Tyrosine (Y) Regulated Kinases). DYRK family members (1A, 1B, 2, 3, 4) share a conserved amino acid sequence Tyr-X-Tyr motif in the activation loop of the catalytic domain and differ in their N- and C- terminal regions. They also have similarities in their functional and chemical characteristics and are distributed in many tissues. DYRK members are highly expressed in testis but little is known about their roles. The aim of this study was to describe DYRK family in three cellular components of the seminiferous epithelium: Sertoli, spermatogonia and spermatocytes and analyze its potential role in the regulation of one of their substrates, muscle glycogen synthase (MGS). Using RT-PCR and Western blot we have detected differential expression of five DYRKs. By subcellular fractionation we observed the distribution of DYRK1A, DYRK1B and MGS and phosphorylated MGS on Ser641. In vitro kinase assay indicated that DYRK1A is active in the three cell lines and the addition of the pharmacological inhibitor harmine dramatically decreased its activity. Additionally, the inhibition of DYRK1A with harmine resulted in a decrease of MGS phosphorylation on Ser641. Preliminary immunoprecipitation assays suggest that there is an interaction between DYRK1A and MGS. Based on these results, we propose that DYRK1A regulates the activity of MGS by phosphorylation, triggering the inhibition of glycogen synthesis, in agreement with the physiological status of the seminiferous epithelium.

Mollusk hemocyanins interacts with the C-type lectin receptors; Mannose Receptor (MR) and Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN), on antigen presenting cells

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Hemocyanins from Megathura crenulata (KLH), Concholepas concholepas (CCH) and Fissurella latimarginata (FLH), have been used as immunostimulants in vaccines against cancer and during immutherapy for superficial bladder cancer. However, the mechanism involved in the positive immunomodulatory effects of these proteins has been scarcely studied. An important feature of hemocyanins is its carbohydrate content, which can reach up to 3% w/w, because these oligosaccharides could be recognized by C-type lectin receptors (CLRs) on antigen presenting cells (APCs), such as MR or DC-SIGN. These innate receptors are involved in classical and alternative antigen presentation on MHC-I and MHC-II. Hence, we proposed CLRs-mediated endocytosis of hemocyanins in APCs triggers alternative processing and presentation pathways. The results demonstrated that: i) Hemocyanins bind to MR and DC-SIGN with high affinity constants, measured by SPR and ELISA. ii) The interaction with MR triggers hemocyanin endocytosis, analysed by an artificial CHO-MR cells system. ii) Murine APCs can uptake hemocyanins, which can be partially inhibited by EDTA and D-Mannose. iv) The cellular destination of FLH and KLH on murine APCs using confocal microscopy, showed localization in Rab5+, Rab7+ or Lamp-1+ compartments. However, CCH was not found in Rab5+ early endosomes, suggesting differential compartmentalization between the hemocyanins. Thus, FLH and KLH could be involved in an alternative processing pathway such as cross-presentation, as supported by an increase in MHC-I and MHC-II expression on murine APCs. We concluded that CLRs have an important role in endocytosis, signalling and alternative processing pathway of hemocyanins on murine APCs.

New Members Session

Exosomes from metastatic breast cancer cells post-interference of ncmtRNAs decreased metastasis in a model of carcinomatosis intraperitonneally

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Introduction Our group has proposed the antisense noncoding mitochondrial RNAs (ASncmtRNAs) as new therapeutic targets for cancer. Knockdown of ASncmtRNAs with a specific antisense oligonucleotide (ASO) causes massive and selective cell death of tumor cells but not normal cells. During intercellular communication, cells release extracellular vesicles such as exosomes. These vesicles, that transport proteins and micro-RNAs, have been implicated in metastasis. This work proposes that exosomes released upon knockdown of ASncmtRNAs have the ability to modulate metastatic properties of the human breast cancer cell line MDA-MB-231 in vivo. Methodology MDA-MB-231 cells were transfected with a therapeutic antisense oligonucleotide (ASO-T) complementary to ASncmtRNA or a non-related ASO (ASO-NR). At 24 h post-transfection, exosomes were purified from supernatants using Exo-spin.Microvesicle characterization was carried out by micro-BCA, western blot, transmission electron microscopy (TEM) and Nanosight analysis. A peritoneal carcinomatosis model was used to evaluate the effect of therapeutic of exosomes derived from ASO-T-treated cells, (Exo-ASO-T) after injecting MDA-MB-231 cells intraperitoneally into NodSciD immunodeficient mice. Then, mice received four intraperitoneal injections of 10 µg exosomes. Finally, at 21 days, total tumor mass in the cavity was examined. Results Migration and Rac activity were inhibited in vitro in cells treated with Exo-ASO-T, compared to controls. The In vivo invasive potential of MDA-MB-231 cancer cells decreased when mice received Exo-ASO-T, while mice injected with Exo-ASO-NR showed increased metastasis. Conclusions Exosomes released from MDA-MB-231 cells upon knockdown of ASncmtRNAs affects tumor communicationand inhibits metastasis.

Transcriptome-wide identification of RNA binding sites of LINE-1 retrotransposon ORF1 protein in human carcinoma cell lines by CLIP-seq

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LINE-1 (L1) sequences comprise 17% of human DNA. They are the only active and autonomous retrotransposons in our genome. L1 retrotransposition requires the formation of a ribonucleoprotein particle containing at least the L1 RNA and the two L1-encoded proteins, ORF1 and ORF2. ORF1 is an RNA-binding protein and ORF2 exhibits endonuclease and reverse transcriptase activities essential for L1 replication. The function of ORF1 in this process is poorly understood. It is hypothesized to assemble preferentially *in cis* on the L1 RNA from which it is translated *in vivo*. To get insight into the function of ORF1 in retrotransposition and in other potential cellular processes, we performed high-throughput sequencing of RNA isolated by crosslinking immunoprecipitation (HITS-CLIP) of endogenously expressed ORF1 in two human carcinoma cell lines. We also analyzed gene expression upon shRNA-mediated reduction of ORF1 levels by RNA-seq. As expected, we identified the L1 RNA as a partner of the ORF1 protein covering all its RNA. Nevertheless, we also identified ORF1-binding sites in many cellular RNAs. The binding *in vivo* of ORF1 protein of L1 retrotransposon to cellular RNAs suggests a role for ORF1 in regulating gene expression at post-transcriptional levels.

Determining the genetic basis of epidermolysis bullosa symptoms through genotype-phenotype associations and ngs

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Among rare diseases, one of the most dramatic examples is Epidermolysis bullosa (EB), also referred as to “butterfly children” due to the extreme skin fragility these patients have. This disorder its characterized by its large genetic and clinical heterogeneity, caused by mutations in 18 genes and resulting in more than 30 different clinical subtypes, which enormously difficult its diagnosis and prognosis specially at the neonatal period where they all look very similar. In this multicentric study, we have used next generation sequencing (NGS) technologies together with a high quality, detailed and extensive clinical evaluation to explore into the genetic basis of EB symptoms. Our cohort expanded to more than 100 Chilean patients from all EB types and clinical evaluations in 4 different health specialty areas: dermatology, pediatrics, ophthalmology and dentistry. Preliminary results from the first two years of the project have already demonstrated population-specific genetic variation with clinical significance. Results obtained from this research will largely contribute to the worldwide understanding of how the genotype influences the phenotype in EB. Moreover, these results will be collected as a comprehensive genotype-phenotype database that will be available for clinicians all over the world, helping them for making a correct diagnosis, deciding how to treat and counsel patients and giving EB patients the chance to aim for a personalized treatment in the future.)

LOOP IIId of the HCV IRES is essential for the structural rearrangement of the 40S-HCV IRES complex.

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As obligatory intracellular parasites, viruses rely on cellular machines to complete their life cycle, and most importantly they recruit the host ribosomes to translate their mRNA. The Hepatitis C viral mRNA initiates translation by directly binding the 40S ribosomal subunit in such a way that the initiation codon is correctly positioned in the P site of the ribosome. Such a property is likely to be central for many viruses, therefore the description of host-pathogen interaction at the molecular level is instrumental to provide new therapeutic targets. In this study, we monitored the 40S ribosomal subunit and the viral RNA structural rearrangement induced upon the formation of the binary complex. We further took advantage of an IRES viral mutant mRNA deficient for translation to identify the interactions necessary to promote translation. Using a combination of structure probing in solution and molecular modeling we establish a whole atom model which appears to be very similar to the one obtained recently by cryoEM. Our model brings new information on the complex, and most importantly reveals some structural rearrangement within the ribosome. This study suggests that the formation of a 'kissing complex' between the viral RNA and the 18S ribosomal RNA locks the 40S ribosomal subunit in a conformation proficient for translation. **Funding**: PICS #5283 – L.I.A - CNRS; Iniciativa Científica Milenio del Ministerio de Economía, Fomento y Turismo: Proyecto P09/016-F; Laboratoire d'Excellence Project ANR-11-LABX-0057-MITOCROSS

Detailed biophysical characterization of the unusual three-state folding and domain swapping of the forkhead domain of human FoxP1

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Three-dimensional domain swapping (DS) is a folding-upon-binding phenomenon where identical structural elements are exchanged between two monomers, breaking stabilizing interactions and reforming them intermolecularly in the process, ultimately leading to an intertwined dimer. Previous studies on DS proteins have shown a high kinetic barrier between monomers and intertwined dimers, but promoting unfolding and refolding at high protein concentrations can favor oligomerization. The forkhead domain of human FoxP transcription factors is an interesting model of DS, because mutations that impair this process are linked to diverse inherited disorders. Moreover, FoxP1 reaches monomer-dimer equilibrium in solution without needing additional perturbations, suggesting that a low kinetic barrier separates both species. However, the current view of DS as a slow process that requires extensive protein unfolding impedes conciliation of its biological occurrence. Here we biophysically characterized the swapped dimer of FoxP1, which is formed within hours, determining that its equilibrium unfolding and dissociation follows a three-state mechanism with a native-like monomeric intermediate, in dramatic contrast with previous DS proteins. We then used hydrogen-deuterium exchange mass spectrometry (HDXMS) to gain further insights of the structural features of the native monomeric and dimeric states of FoxP1 and of its folding intermediate. Our results show that both the monomeric and dimeric forms are characterized by a high solvent accessibility in its β sheet structure (strands β 1-3), but are further loosened at chaotropic concentrations where the intermediate is highly populated. Our results indicate that this region could be important to explain the unusual DS mechanism of FoxP1.

Deciphering gene regulatory networks involved in the crosstalk between sulfur and nitrogen metabolism in *Arabidopsis thaliana.*

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Our research is focused on the molecular mechanisms underlying the response of plants to nutrients, specifically, on the complex interactions between nutrients in metabolic pathways. Plant growth is limited by the availability of nutrients in the soil, particularly by nitrogen and sulfur, which are required in large amounts for plant growth. Although there have been significant advances into understanding how plants sense nutrients in their environments and transduce this information to the whole plant, most studies have dealt with only a single nutrient. In nature, however, plants must contend with soils that have variable relative amounts of different nutrients. An interaction between sulfate and nitrate assimilation pathways has been well documented and is an attractive model for studying the plant responses to complex changes in nutrients availability. Deficiency of either nitrogen or sulfur reduces the uptake and assimilation of the other. This phenomenon is mainly due the fact that nitrogen and sulfur are assimilated by plants primarily into protein. Therefore, the plant requires a mechanism to coordinate and balance the assimilation of these essential components to satisfy the requirements of net protein synthesis. I will present and discuss work done in my lab to understand the crosstalk between between sulfur and nitrogen metabolic pathways.

The unexpected structure of the designed protein Octarellin V.1 forms a challenge for protein structure prediction tools

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Despite impressive successes in protein design, designing a well-folded protein of more 100 amino acids *de novo*remains a formidable challenge. Exploiting the promising biophysical features of the artificial protein Octarellin V, we improved this protein by directed evolution, thus creating a more stable and soluble protein: Octarellin V.1. Next, we obtained crystals of Octarellin V.1 in complex with crystallization chaperons and determined the tertiary structure. The experimental structure of Octarellin V.1 differs from its *in silico* design: the (αβα) sandwich architecture bears some resemblance to a Rossman-like fold instead of the intended TIM-barrel fold. This surprising result gave us a unique and attractive opportunity to test the state of the art in protein structure prediction, using this artificial protein free of any natural selection. We tested 13 automated webservers for protein structure prediction and found none of them to predict the actual structure. More than 50% of them predicted a TIM-barrel fold, i.e. the structure we set out to design more than 10 years ago. In addition, local software runs that are human operated can sample a structure similar to the experimental one but fail in selecting it, suggesting that the scoring and ranking functions should be improved. We propose that artificial proteins could be used as tools to test the accuracy of protein structure prediction algorithms, because their lack of evolutionary pressure and unique sequences features.

Epigenetic regulation of Senescence-associated genes in hippocampal neurons

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Introduction: Senescence is a form of terminal differentiation characterized in dividing cells that requires activation of senescence-associated (SA) genes. During the senescence process, It has been described the participation of Polycomb-Group proteins (PcG) complexes. PcGs are key epigenetic regulators that can silence genes through deposition of H3K27me3 on target promoters. However, it is unknown if the senescence program is maintained in non-dividing cells such as mature neurons. **Methodology:** Expression of PcG proteins and SA genes in rat hippocampal tissue (E18, 1m, 3m and 18m) and culture neurons (5, 15 and 25 DIV) were analyzed by qRT-PCR, western-blot and immunostaining. Senescence process was analyzed by enzyme-activity associated to senescence. ChIP assays were performed with specific antibodies against PcG proteins and histone H3 post-translational modifications and quantified by qPCR using specific primers to senescence-associated genes. **Results:** Expression of SA genes increased concomitant to enzyme-activity associated to senescence. PcG-Ezh2 protein is highly expressed in embryonic stages and decreased dramatically during neuronal maturation. On the other hand, PcG-Ezh1 protein maintains a high expression through development. PcGs are present differentially on senescence-associated genes: Ezh2 in the embryonic stage when genes are not expressed while that Ezh1 is in a mature state where senescence genes are expressed. **Conclusions**: Our findings suggest that in young neurons senescence-associated genes are transcriptionally repressed by PcGs proteins, specifically by Ezh2-PcG complex. With maturation these genes get expressed by an epigenetic mechanism involving PcG-Ezh1 complexes.

Cortisol-mediated non-genomic signaling in the stress response of teleost

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Introduction: Salmonids reared under intensive conditions are exposed to stressors, which negatively impacts growth. In teleosts, cortisol is the primary glucocorticoid and an essential regulator of the neuroendocrine response to stress. Glucocorticoids exert their effects predominantly through their interaction with a cytoplasmic glucocorticoid receptor and by modulating the expression of stress response genes. This mode of action is referred to as the genomic pathway. Additionally, glucocorticoids also have actions that are too rapid to be mediated by the genomic pathway and are referred to as the non-genomic pathway. While cortisol-mediated genomic signaling in response to stress has been extensively studied in salmonids, very little is known about non-genomic signaling and its relevance in the stress response.

Methods: Through *in vitro* approached we assessed the effects of stress mediated by cortisol and cortisol-BSA (a membrane-impermeable agent) in the generation of reactive oxygen species (ROS) in rainbow trout (*Oncorhynchus mykiss*) skeletal muscle. These ROS were not suppressed by inhibitors of transcription or translation, thus suggesting a non-genomic mechanism. To elucidate the mechanisms and the key players involved in this non-genomic response, we used a proteomic and transcriptomic approach (RNA-seq).

Results and conclusion: Our results show that non-genomic signaling is associated to activation of signaling pathway involved the expression of genes related to energy metabolism and antioxidant activity. Based on these data, we conclude that cortisol-mediated non-genomic mechanism associated with a membrane glucocorticoid receptor, contributes to the activation and expression of an antioxidant network as a compensatory mechanism to stress.

Dual regulation of Abeta levels by Presenilin 1

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Alzheimer's disease (AD) is characterized by accumulation of the Abeta peptide, which is generated through sequential proteolysis of the Amyloid Precursor Protein (APP), first by the action of beta-secretase, generating beta-CTF, and then by the gamma-secretase complex, generating Abeta. Gamma-secretase is an intramembranous protein complex composed of Aph1, Pen2, Nicastrin and Presenilin 1 (PS1). Although it has a central role in the pathogenesis of AD, knowledge of the mechanisms that regulate PS1 function is limited. Here we show that phosphorylation of PS1 has a dramatic effect on Abeta levels *in vivo*, although it does not affect gamma-secretase activity. We identified CK1 as the endogenous kinase responsible for the phosphorylation of PS1. Inhibition of CK1 leads to a decrease in PS1 phosphorylation and to an increase in Abeta levels in cultured cells. Transgenic mice in which the phosphorylated residue of PS1 was mutated to Ala show dramatic increases in the levels of Abeta peptide and in beta-CTF *in vivo*. Finally, we show that this mutation impairs the autophagic degradation of beta-CTF, resulting in its accumulation and increased levels of Abeta peptide.

Our results demonstrate that PS1 regulates Abeta levels by a unique bi-functional mechanism. In addition to its known role as the catalytic subunit of the gamma-secretase complex, selective phosphorylation of PS1 also decreases Abeta levels by increasing beta-CTF degradation through autophagy. Elucidation of the mechanism by which PS1 regulates beta-CTF degradation may aid in the development of potential therapies for Alzheimer's disease.

Posters Session

1) MONOMER-DIMER SWITCHING: PSYCHROPHILIC ADP-DEPENDENT KINASE DIMERIZATION AS AN ENERGY SENSOR IN GLYCOLYTIC PATHWAY OF ARCHAEA

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In the glycolytic pathway of Bacteria and Eukarya the enzymes with glucokinase (GK) and phosphofructokinase (PFK) activity use MgATP as the phosphoryl donor. In many archaea, these two glycolytic activities are performed by bifunctional enzyme that uses MgADP to phosphorylate glucose and fructose-6P. These enzymes also present glucose-6P phosphatase activity with MgAMP in the gluconeogenic pathway. Interestingly, in this work, studies with the psychrophilic enzyme ADP-PFK/GK from *M. burtonii* through small-angle X-ray scattering showed that the enzyme is a monomer in the presence of MgAMP (gluconeogenic pathway), whereas in presence of MgADP and MgATP the enzyme is a dimer (glycolytic pathway).This suggest that the enzyme could be able to select its function in a given metabolic pathway by changes in its oligomerization state. Dimer functionality was assessed by intrinsic fluorescence measurements and enzyme kinetics. Binding of MgADP, MgATP and ADP^{-4} determined by intrinsic fluorescent measurements show that the former two, besides forming dimers, display negative cooperativity while ADP^{-4} binds with positive cooperativity. To evaluate the impact of this phenomenon on enzyme activity kinetics measurements pre-incubating the enzyme at different ADP^{-4} and MgADP concentrations were performed. The results showed that only ADP^{-4} and not the substrate of the enzyme (MgADP) produce an increase in the GK but not in the PFK activity. The complex interplay between the oligomerization state and activation of one if its specific activity pointed out at this enzyme as key regulatory point in the operation of either the glycolytic or the gluconeogenic pathway.

2) DIFFERENTIAL EXPRESSION OF METABOLISM-RELATED GENES IN LIVER AND SKELETAL MUSCLE OF FINE FLOUNDER (*PARALICHTHYS ADSPERSUS)* UNDER CHRONIC STRESS.

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Introduction: Liver and skeletal muscle are fundamental to maintain the metabolic homeostasis in vertebrates under stressing conditions. Thus, during the early stress response, the carbohydrates metabolism is increased to obtain energy and overcome this situation. However, particularly in inferior vertebrates, such as fish, the regulation of carbohydrates metabolism, as well as other metabolic adjustements derived of chronic stress response are not well established. In this context, we evaluated the expression of key components involved in the metabolism of carbohydrates, lipids and proteins in liver and skeletal muscle tissues of Fine flounders subjected to long periods of confinement stress. **Materials and Methods:** Fish were maintained in four tanks, two experimental (17,3 Kg/m^3) and two controls (6,7 Kg/m^3). After 4 and 7 weeks of treatment, RNA from liver and skeletal muscle tissues were collected from 6 individual/tank. RT-qPCR was used to evaluate metabolism-related gene expression reprograming associated to high density farming conditions. **Results:** We determined that confinement stress modulates the liver gene expression of key enzymes related to carbohydrates metabolism in a time-dependent way, such as: *glucose-6-phosphatase*. In addition, other relevant participants involved in metabolism exhibit differential expression patterns in both, liver and skeletal muscle tissues: *glucocorticoids receptor*, *cathepsin D*, *hormone-sensitive lipase1* and *arginase II,* among others. **Discussion:** The confinement stress modulates differentially the expression of metabolism-related genes in liver and skeletal muscle of Fine flounder. Further functional experiments are necessary to reveal the role of these genes in chronic stress response in fish.

3) EQUILIBRATIVE NUCLEOSIDE TRANSPORTER 2 IS A TARGET OF INSULIN/PI3K/AKT SIGNALING AND DYSREGULATED IN DIABETIC GLOMERULOPATHY.

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INTRODUCTION. Deficient insulin signaling has been proposed as a key event mediating diabetic glomerulopathy. Additionally, diabetic kidney disease has been related to increased levels of adenosine. Therefore, we tested a link between insulin deficiency and dysregulated activity of the equilibrative nucleoside transporters (ENTs) responsible for controlling extracellular levels of adenosine.

METHODS: Rat glomeruli ex vivo and primary cultured podocyte were used. The transport kinetic parameters for the ENT2 protein in podocytes exposed to 5 and 25 mM glucose and 10nM insulin were determined. Western blot assays were performed to determine insulin signaling pathways affecting ENT2 expression and activity in podocytes and ENT2 levels in diabetic rats. Contents of adenosine were evaluated by HPLC.

RESULTS: In ex vivo glomeruli, high D-glucose decreased nucleoside uptake mediated by ENT1 and ENT2 transporters, resulting in augmented extracellular levels of adenosine. This condition was reverted by exposure to insulin, upregulating ENT2 activity. The effects of insulin on ENT2 were mediated by insulin receptor signaling through PI3K/AKT pathway and independent of MAPK activation. Insulin was able to increase ENT2 V_{max} promoting ENT2 translocation to plasma membrane. Also, PI3K activity was necessary to maintain ENT2 protein levels. In glomeruli of streptozotocin-induced diabetic rats, insulin deficiency leads to decreased activity and content of ENT2 and increased levels of adenosine.

CONCLUSION: We evidenced ENT2 as a target of insulin and sensitive to dysregulation in diabetes, leading to chronically increased nucleoside levels and thereby setting conditions conducive to kidney injury.

4) INVOLVEMENT OF VPG PROTEIN DURING THE TRANSLATION OF MRNAS FROM INFECTIOUS PANCREATIC NECROSIS VIRUS (IPNV)

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Aquaculture is the second most important economic activity in our country. However, this sector has been subject of ongoing threat by several pathogenic agents, such as bacteria and viruses. One of the viruses that affect the Chilean industry is the Infectious Pancreatic Necrosis Virus (IPNV), which is spread widely among salmon farms in the country. IPNV belongs to the genus *Aquabirnavirus*, of the family *Birnaviridae*. The birnavirus is unenveloped, and its genome is composed by two uncapped and unpolyadenilated dsRNA segments. Segment A has two ORFs, which encodes for structural and non-structural proteins. Segment B encodes for the RNA viral polymerase RNA-dependent, VP1, which is involved in gRNA replication and mRNA transcription. VP1 is also bonded covalently to each 5' end of these two gRNA segments, becoming Vpg. This investigation achieves to elucidate the involvement of VPg during the infectious process, particularly in the assembly of the translational machinery. In order to do this, we expressed recVP1 protein, with a conserved activity, similar to the native protein in IPNV, able to self-guanylate in a Ser, and to synthesize viral RNA. The interaction between recVP1 protein and selected translation initiation factors (eIFs) was analyzed through immunoprecipitation and pull-down assays. Results obtained indicate that VP1 interacts directly with eIF4E, the initiation factor that recognizes Cap during canonical translation of cellular mRNAs, but it is not able to interact with eIF4G. These outcomes might suggest that VPg would be involved in the translation of viral mRNAs.

5) NEW METHOD FOR MICROSOMES PRODUCTION FROM *SACCHAROMYCES CEREVISIAE* FOR THE STUDY OF THE MECHANOCHEMICAL MECHANISM OF BIP

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Posttranslational translocation (PT) of proteins through the endoplasmic reticulum (ER) is mediated by both a channel protein called Sec61 and auxiliary motors proteins such as BiP. BiP is member of the family of ATPase Hsp70 type chaperones, and studies suggest that it would be involved in the force application during the process of PT of the extended polypeptide. The work exerted by BiP in the translocation of protein is not clear; however studies suggest that BiP could be involved in a "*ratchet*" passive mechanism and/or a mechanism direct active of pulling. Microsomes are small vesicles resulting from the fragmentation of ER when cells are homogenized, and are very important for the protein translocation assays. In this study we combine two conventional methodologies to obtain pure microsomes (Parodi, J.B.C, 1994, 269, 30701; Sheckman, J.Cell.Biol., 1995, 131, 1161). One of the differences between both methodologies is how homogenize the cells, Schekman method (SM) used a lyticase enzyme and as a last step a sucrose gradient obtaining purified microsomes; in the Parodi method (PM) glass beads are used to obtain a microsomal fraction. Our method uses glass bead as PM and as a last step a sucrose gradient (SM). Our yield of purified microsomes is 0.152 µg/L of culture of *Sacharomyces cerevisiae* yeast. When it used the conventional methodologies of SM is obtain 4.2 µg/L of culture. We reduce costs and time of production. These microsomes will be used to determine the mechanochemical force of BiP by assays in bulk.

6) ACTIVATION OF MUSCULAR ATROPHY SIGNALING PATHWAYS IN THE FINE FLOUNDER (*PARALICHTHYS ADSPERSUS*) AFTER A *V. ORDALII* INFECTION.

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Introduction: The fine flounder is an endemic flatfish with great potential for aquaculture diversification. Despite its quality, an unwanted characteristic of this fish is its remarkable slow growth, which is also negatively affected by factors such as fasting, handling and confinement. Our group recently showed that these factors could promote the muscle atrophy by two main systems related to protein degradation: the ubiquitin proteasome system (UPS) and autophagy (ALS). However, other factors like pathogens infections can also have negative effects over the growth. In this context, we evaluated the effect of an *in vivo* bacterial infection over the muscle atrophy mechanisms.

Methodology: Fish were randomly allocated in groups (20 individuals/tank) and acclimatized prior to the challenge. One group was bathed in seawater containing 2.59 x 10^7 CFU/ml of *V. ordalii* Vo-LM-18 strain. Control groups were bathed in water with un-inoculated broth, or left untreated. Individuals were removed from each group after 2, 4 and 10 days post-infection, and skeletal muscle samples used for qPCR and proteomic analysis.

Results: *V. ordalii* was detected in all infected samples by qPCR. We observed an overexpression of genes related with atrophy of the muscle fiber. Infected fish showed no changes in the mRNA levels of the E3-ubiquitin ligases *murf1* and *atrogin1* (ubiquitin proteasome system). Moreover, the ratio LC3-II/LC3-I was increased in the entire trial, suggesting the activation of the autophagy pathway.

Conclusions: Taken together, these results suggest the activation of muscle wasting pathways, through the ALS system, as a response of the bacterial infection.

7) ROLE OF S6K1 ON INSULIN-DEPENDENT MITOCHONDRIAL MORPHOLOGY AND FUNCTION IN CARDIOMYOCYTES

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(Sponsored by FONDAP 15130011 (SL), FONDECYT 1161156 (SL). IG Hold A PhD Fellowship From CONICYT.)

The myocardium requires a continuous and abundant supply of ATP. Due to this high energy demand, mitochondria play an essential role to achieve this requirement. The morphology and function of this organelle is dynamically changing. This implies that the mitochondria are melted and divided depending on the metabolic needs of the cell. Particularly, mitochondrial fusion is complex, because it must melt both outer and internal membranes, which are regulated by Mfn1/Mfn2 and OPA1, respectively. Our recent work showed that insulin increased mitochondrial fusion by activating the signaling pathway Akt/mTORC1/OPA1 in cardiomyocytes. However, it remains unclear which is the effector downstream of mTORC1 that allows such changes. Because mTORC1 phosphorylates and activates S6K1, we test the hypothesis here that S6K1mediates the increase in OPA1. To this end, cardiomyocytes were treated with insulin 10 nM for 3h. As previously shown insulin increased OPA1 levels, oxygen consumption, mitochondrial membrane potential and promoted mitochondrial fusion in cultured cardiomyocytes. However, all these effects were abolished when the cells were pretreated with a chemical inhibitor for S6K1 before exposure to insulin. The upstream effector of insulin signaling pathway Akt was not altered by the S6K1 inhibitor. Also, when cells were transfected with an siRNA against S6K1, there was no change in OPA1 levels in response to insulin. These results suggest that S6K1 could mediate the effects of insulin on mitochondrial function and morphology in cardiomyocytes.

8) DIFFERENTIAL MIRNAS EXPRESSION ASSOCIATED TO HYPER-RADIOSENSITIVITY TO LOW DOSES OF IONIZING RADIATION IN DLD-1 CELLS.

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Introduction Conventional radiotherapy protocols for treatment of colorectal cancer consist of administration of high doses ionizing radiation (IR), which is associated to several side effects. Recently, a phenomenon known as Hyper-Radiosensitivity to Low Doses (HRSLD, <1Gy) has been described and appears as an opportunity to decrease radiation exposure while maintaining therapeutic success. Here we evaluate the miRNA expression associated to low dose IR in order to elucidate the underlying mechanism of HRSLD. **Methodology** Human colorectal cancer cells (DLD-1) were irradiated with low doses of IR (0.6 Gy) and cell death was evaluated by MTS, trypan blue exclusion, Caspase 3/7 and $SubG_1$ assays. DNA damage was assessed by γH2AX staining. Expression of 80 miRNAs was determined in a array-PCR format. Validation of targets was performed by qRT-PCR. **Results** DLD-1 cells display HRSLD when exposed to 0.6 Gy of IR. Expression profiling identified 4 over-expressed (miR-2053p, miR1, miR338-3p and miR2045p) and 3 downregulated (miR1225p, miR5423p and miR1345p) miRNAs in irradiated cells. Target validation by RT-qPCR confirmed these findings. Although DNA damage was induced, miRNA profile at 0.6 Gy were different than the one from cells exposed to high-dose ionizing radiation (12 Gy), suggesting a distinct low dose radiation-induced cell death mechanism. **Conclusions** Irradiation of DLD-1 cells using low doses (0.6 Gy) induces DNA damage, apoptosis and a differential expression of miRNAs. Further analysis of distinct miRNA´s target will provide valuable insights into the mechanism associated to the HRSLD.

9) *DE NOVO* ASSEMBLY AND CHARACTERIZATION OF THE CHILEAN RED SEA URCHIN (*LOXECHINUS ALBUS*) TRANSCRIPTOME USING NEXT GENERATION SEQUENCING

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Introduction: The red sea urchin (*Loxechinus albus*) is an endemic echinoderm species distributed along the Chilean coasts. This resource has been over harvested in recent years depleting their natural populations. At present, there are few reported gene sequences available in public databases, restricting the molecular study in this species and the establishment of breeding programs based on molecular markers. The aim of this study was to present the first annotated reference transcriptome of *L. albus*, using NGS technologies.

Methods: Total RNA was extracted from gonad, intestine and coelomocyte fluid of juvenile red sea urchin. The samples were sequenced in paired-end using Miseq technology (Illumina). Raw data was filtered using flexbar software and assembly de novo with Trinity. The high-quality reads were mapped with bowtie2 software and filtered with Corset. Finally, we detected transcripts with differential expression using edgeR program.

Results: The RNA sequencing generated 91,119,300 high quality paired-ends reads, which were de novo assembled producing 278,803 contigs, creating a reference transcriptome with a N50 of 1,418. Subsequently, were obtained 185,239 filtered contigs with a N50 of 1.769 bp, grouped in clusters. Furthermore gene onthology analysis revealed notable differences in the expression profiles between gonad, intestine and coelomocyte fluid, allowing the detection of transcripts associated to specifics biological processes.

Conclusions: This dataset represents the first transcriptomic resource for the red sea urchin, providing a rich supply of data for discovering and identifying genes associated to physiological processes in this species. Our work provides an invaluable transcriptomic resource for future gene expression analysis.

10) *TESMIN/TSO1-LIKE CXC 5* AFFECTS SHOOT DEVELOPMENT AND IS A DIRECT TARGET OF GCN5 IN ARABIDOPSIS.

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Proteins involved in chromatin remodelling control the plants and animals development through direct regulating the expression of specific developmental transcription factors. GENERAL CONTROL NON-REPRESSIBLE 5 (GCN5) is a subunit of SAGA complex and important histone acetyltransferase required for gene expression in eukaryotes. Mutations in arabidopsis GCN5 show various pleiotropic defects during plant development. In this work, we have identified a set of transcription factors as direct targets of GCN5 in arabidopsis. Chromatin immunoprecipitation assays indicated that GCN5 protein is enriched at the promoter regions of Tesmin/TSO1-like CXC 5 (TCX5). This gene is one of the plant homologues to the human TESMIN gene, originally identified by its specific expression in testes and ovary development. There is evidence of the role of TSO1 in flowers and pollen development in plants. A TCX5 reporter plant shown its expression in the hypocotyl vasculature and shoot apical meristem. The expression of TCX5 is repressed in a GCN5 loss-of function plant. Mutant alleles of TCX5 are dwarf with morphological defects in the shoot that resembles the phenotype observed in GCN5 mutant plant. These results suggest an important role of TCX5 in plants and further experiments will reveal the genetic interaction of TCX5 with members of SAGA complex in the control of plant development.

11) IDENTIFICATION AND CHARACTERISATION OF TWO PUTATIVE LIPOYL SYNTHASES (LIP1) IN SOLANUM LYCOPERSICUM (TOMATO)

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Lipoic acid (LA) is a functional and structural metabolite with powerful antioxidant capacities present in eukaryotic and prokaryotic organisms. LA is both lipid- and water-soluble, and is the prosthetic group in a number of key multi-subunit enzymes complexes, including pyruvate decarboxylase and α-ketoglutarate dehydrogenase. LA synthesis and incorporation into these proteins (lipoylation) proceeds via a de novo or a salvage pathway. During de novo synthesis, octanoyl transferase (LIP2) uses recently-synthesised octanoyl groups linked to the acyl carrier protein to transoctanylate target proteins. Subsequently, lipoyl synthase (LIP1) catalyses the final step by inserting two sulphur atoms into the prosthetic group. Whilst a number of the enzymes have been functionally-characterised in Arabidopsis thaliana, the aim of the current work is to identify and evaluate the role of this pathway in a fruit-bearing species. Towards this aim, we identified two proteins in tomato (Solanum lycopersicum) with the molecular characteristics of LIP1, which we call SlLIP1 and SlLIP1p, which possess 84% and 78% amino acid identity with AtLIP1. Confirming bioinformatic predictions, Sllip1p has a plastidial localisation whereas Sllip1 is mitochondrial, as shown by confocal microscopy. Experiments to determine the molecular function of both proteins are underway, by functional complementation of a bacterial mutant, and their stable over-expression in *A. thaliana* and tomato. Funding: Conicyt Anillo ACT-1110 (to MH), and Conicyt Doctoral (21160916 to JA) and Master (22151178 to SM) scholarships.

12) FUNCTIONAL GENOMICS AND MOLECULAR STUDIES OF NON-CAROTENOGENIC GENES INVOLVED IN SYNTHESIS AND ACCUMULATION OF CAROTENOIDS IN *DAUCUS CAROTA* UNDER DARK/LIGHT CONDITIONS

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In plants, carotenoids are pigments involved in photosynthesis, photo-oxidative-damage protection and phytohormone synthesis. Animals must acquire them through dietary intake as they are precursors of vitamin A. During plant development, light induces carotenogenic gene transcription and the synthesis of carotenoids in leaves, flowers and fruits. The orange variety of *Daucus carota* (carrot) produces carotenoids, principally α- and β-carotene, in the storage root in dark at concentrations that are unique among plants. Light inhibits carotenoids synthesis in carrot storage root, as well as the expression of carotenogenic genes. At present, the contribution of non-carotenogenic master genes in carotenoid synthesis and/or accumulation remains to be determined. With the aim to find and characterize non-carotenogenic genes involved in the synthesis and/or accumulation of carotenoids in carrot storage root, we performed RNA-Seq analysis from roots grown in dark and light conditions. Thus, we obtained a reference transcriptome with 63,124 contigs using *de novo* assembly, from which 18,488 contigs are differentially expressed (DEG) between the two experimental conditions. Approximately 75 contigs belong to genes involved in light perception, 122 in hormone response, and about 198 might correspond to transcription factors. We report the enrichment analysis of GO terms with DEGs and the validation of the transcriptome assembly through qPCR. Genes such as *Phytochrome-Rapidly-Regulated 1 (Par1)* related with the Shade-Avoidance-Syndrome and the chromoplast differentiation *Orange* (*Or*) gene are expressed in dark roots. The *Par1* over-expression in carrot actually produces orange embryo and seedlings, demonstrating that *Par1* positively regulates *in vivo* the carrot carotenoid synthesis.

13) ACTIVIDAD CITOTÓXICA Y PRO-APOPTÓTICA DE EXTRACTOS MICROALGALES SOBRE LA LÍNEA CELULAR LNCAP DE CÁNCER DE PRÓSTATA HUMANO.

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El cáncer es la principal causa de muerte a nivel mundial, los tratamientos actuales son costosos, invasivos y no siempre efectivos. Esto hace necesario la búsqueda de nuevas alternativas terapéuticas, más selectivas, menos agresivas y tóxicas para los pacientes.

En la actualidad los compuestos obtenidos a partir de productos naturales se potencian como importantes alternativas para la generación de drogas con efecto anticancerígeno. En este contexto, se ha descrito especies microalgales como fuente de obtención de metabolitos bioactivos, con potencial terapéutico, asociado a la capacidad de generar efecto citotóxico y antiproliferativo sobre células cancerígenas.

El objetivo de este trabajo fue evaluar el efecto citotóxico y la actividad pro-apoptótica de 3 especies microalgales, *Nannochloropsis oculata, Tetraselmis suecica y Chlamydomonas reinhardtii* sobre la línea celular LNCaP derivada de cáncer de próstata humano, y comparar estos parámetros respecto a la línea no cancerígena humana derivada de riñon de embrión humano Hek 293.

Distintas concentraciones (50 a 1000µg/ml) de extractos obtenidos con diferentes solventes (etanol, metanol y diclorometano), fueron evaluados respecto a: 1) actividad citotóxica sobre ambas líneas celulares, mediante un ensayo colorimétrico con reactivo MTS, 2) actividad pro-apoptótica a través de ensayo de TUNEL y detección de caspasa-3, y 3) determinación de perfiles mediante HPLC.

Los mejores resultados de citotoxicidad se obtuvieron para extractos etanólicos de *C. reinhardtii*. En células cancerígenas, éstos presentaron diferencias significativas respecto a las células control. Además, presentaron efecto citotóxico significativamente mayor sobre estas células comparado con aquellas no cancerigenas.

14) ADAR1 PROMOTES ACTIVATION OF THE WNT/β-CATENIN PATHWAY IN BREAST CANCER.

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The enzyme ADAR1 (Adenosine deaminases Acting on RNA 1) is one of the most expressed genes in breast cancer. ADAR1 overexpression is associated with poor patients prognosis in several malignancies. β-catenin and GSK-3β are among ADAR edited candidate target RNAs, suggesting a possible association of ADAR1 activity with the Wnt/β-catenin signaling. This led us to investigate the effect of ADAR1 on this specific signaling pathway.

ADAR1 (p110 isoform) expression in different breast cancer cell lines was assessed. ADAR p110 was overexpressed in the breast cancer metastatic cell line MDA-MB-231 by lentiviral transduction. Total protein extracts were analyzed by western blot to study the protein levels of ADAR1, β-catenin, GSK-3β, and targets of the Wnt/β-catenin pathway. Besides, the activation of the Wnt/β-catenin pathway was determined using the TOP/FOP luciferase reporter assay.

The expression of isoform ADAR1 p110 is low in the cell line MDA-MB-231 compared to non-tumoral MCF-10A cells, and its overexpression is associated with a decreased of GSK-3β, an increases in the expression of Wnt/β-catenin targets and an increase in the activation of TCF/LEF (measured by the TOP/FOP luciferase reporter assay).

Our results lead us to propose ADAR1 p110 as a new regulator of the Wnt/β-catenin signaling pathway, suggesting a role of for ADAR1 p110 in breast cancer progression.

15) IDENTIFICATION OF PROTEIN SUPERFAMILIES SIGNIFICANTLY MUTATED IN CANCER

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Domains are structural, functional and evolutionary modules of proteins. They are an important level of classification at which to study cancer mutations, the literature around cancer mutations in domains is scarce. In this study we aimed to identify which protein superfamilies of domains have a high incidence of significantly mutated genes (SMGs) in tumor exomes, so we can then use the known phenotypes resulting from mutations of one member of the superfamily to understand the effects of analogous mutations in other members of the superfamily. We combined the information from a database of significantly mutated genes in cancer (MutSig) with that of the superfamily assignments of the domains present in the human proteome (SUPERFAMILY). In each tumor type and for each protein superfamily, we obtained the percentage of SMGs among the genes of the superfamily (%SMG). To determine which superfamily had a higher %SMGs than expected by chance, a null probability distribution of SMGs for each superfamily was calculated, with the number of SMGs in a genome set as the number of SMGs identified for each type of tumor. Of the superfamilies that had more SMGs than expected by the null hypothesis, we identified the isocitrate/isopropylmalate dehydrogenase like superfamily, in which their SMGs had a conserved mutation resulting in the same phenotype. Additional theoretical work has allowed us to better understand the mechanism of action of this superfamily in general, and the impact of the conserved mutation in particular. This work is supported by FONDECYT de Iniciación Nº 11130578, awarded to Daniel Almonacid.

16) TRANSCRIPTOME ANALYSIS OF TUMOR CELLS TREATED WITH OLIGONUCLEOTIDES TARGETED TO THE ANTISENSE NONCODING MITOCHONDRIAL RNA.

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The study of the transcriptome of tumor cells has elucidated a central role for the vast noncoding landscape of the human genome in tumorigenesis. Our laboratory has described and characterized a new family of non-coding RNAs of mitochondrial origin (ncmtRNAs) composed by Sense (SncmtRNA) and Antisense (ASncmtRNAs) versions. Through different techniques such as *in situ* hybridization (ISH), northern blot and RT-PCR, our laboratory has shown that SncmtRNA and ASncmtRNA are expressed in normal proliferating cells, whereas in tumor cells, ASncmtRNA is downregulated. Knockdown of ASncmtRNA with specific antisense oligonucleotides (ASO) causes massive apoptotic death of a wide array of tumor cells lines. Apoptosis is preceded by a drastic reduction in proliferative index. The same treatment applied to normal cells caused no effect on cell viability, suggesting treatment selectivity. The interest of our group is focused on the molecular mechanisms that trigger the selective apoptosis of tumor cells, particularly with respect to changes in the expression of various genes involved in the regulation of the cell cycle. Transcriptome and proteome analysis were carried out in the human breast adenocarcinoma cell line MDA-MB-231, treated with specific ASO against the ASncmtRNA, compared to an unrelated control ASO and untreated cells. At present, we have found several genes that are differently expressed at the mRNA and/or protein levels, such as Survivin, Cyclin B1, Cyclin D1 and SertaD1, among others. The complete analysis will shed lights on the mechanisms involved.

17) *IN SILICO* PREDICTION OF BIOLOGICAL TARGETS OF SMALL MOLECULES BY A MOLECULAR FINGERPRINT APPROACH

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Experimental profiling of drug candidates against a large panel of diverse biological targets is cost and time intensive. Computational methods for target identification are useful to predict off-targets, for drug repositioning and to deorphanize ligands without known targets and receptors without known ligands. Repositioning of known drugs has become especially attractive with rising drug development costs. Here, we present a chemical similarity approach to biological target prediction. Chemical similarity searching is a type of ligand-based approach based on the frequent observation that structurally similar compounds have similar physicochemical properties and possibly similar biological profiles. Small molecules were represented by Mold^2 topological descriptors and Open Babel's path-based FP2 fingerprints. Mold^2 descriptors were standardized. Target proteins were predicted by estimating the chemical similarity of query molecules to annotated ligands of biological targets. We validated our approach with a publicly available dataset of 2070 drug-protein interactions derived from ChEMBL. Results were analyzed by receiver operating characteristic (ROC) analysis and leave-one-out cross-validation. We obtained average area under the ROC curve (AUC) values of 0.971 for Mold^2 descriptors, 0.974 for Open Babel fingerprints, and 0.980 when combined by logit regression. These results indicate that our chemical similarity method is well suited for target prediction. In summary, we present a fast, structure-independent approach for biological target prediction with straightforward application in drug repositioning.

18) HIGH CONTENT SCREEN IDENTIFIES THE PSMD14 DEUBIQUITINATING ENZYME AS A NOVEL REGULATOR OF AMYLOID PRECURSOR PROTEIN (APP) METABOLISM

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Introduction: Alzheimer's disease (AD) is the most prevalent age-related neurodegenerative disorder, has been associated with high intracellular levels of the C-terminal fragment β (C99) produced through cleavage of Amyloid Precursor Protein (APP) by BACE1, which contributes to long-term memory impairment, a pathological hallmark of AD. In a previous work we found that ubiquitination of C99 is required for its proteasomal clearance. Therefore to gain insight the role of the Ubiquitin-Proteasome System (UPS) we analyzed systematically the contribution of a large Ubiquitin-related selection of genes known as the *Ubiquitome* in the turnover of APP and C99. **Methodology:** H4 neuroglioma cells stably expressing the reporter APP-EGFP were used for a High-Content siRNA screen using a library of ~1200 ON-TARGETplus SMARTpool siRNAs. The screen images were acquired by an INCell2000 automatic microscope and either vesicles or total cell fluorescent intensity were quantified in over 1500 individual cells per gene. High and low hits were deconvolved into its four individual siRNA duplexes. Efficiency of the Knockdown (KD) of selected hits was validated by RT-qPCR and immunoblot. Further their cellular effects were tested with APP and C99 endogenous levels. **Results and Conclusions:** This study identified several hits directly involved in proteostasis control. Among them PSMD14 was selected as a major regulator of APP/C99 levels. PSMD14 is a subunit of the 26S proteasome and display deubiquitinase activity, which removes ubiquitin chains of substrates during proteasomal degradation. Our results reveal that UPS plays a crucial role in APP metabolism elucidating a key new regulator of APP metabolism.

19) EXOSOMES RELEASED UPON KNOCKDOWN OF THE ANTISENSE NON-CODING MITOCHONDRIAL RNAS MODULATE TUMORIGENIC PROPERTIES OF THE HUMAN BREAST CANCER CELL LINE MDA-MB-231.

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Introduction Breast cancer is the most deadly female cancer worldwide; however, therapies today are insufficient to eradicate this disease due to tumor heterogeneity. Our group has proposed a new therapeutic target corresponding to a family of long non-coding mitochondrial RNAs (ncmtRNAs) called Sense (SncmtRNAs) and Antisense (ASncmtRNAs). Knockdown of ASncmtRNAs causes massive and selective cell death in tumor cells but not in normal cells. During intercellular communication, cells release extracellular vesicles such as exosomes that contain proteins and RNAs with the potential to influence recipient cells' proliferation and/or trigger apoptosis. This work proposes that exosomes released post-knockdown of ASncmtRNAs have the ability to modulate tumorigenic properties of the human breast cancer cell line MDA-MB-231 *in vitro*. **Methodology** MDA-MB-231 cells were transfected with a therapeutic antisense oligonucleotide (ASO-T) complementary to ASncmtRNA or a non-related ASO (ASO-NR) using Lipofectamine2000. At 24h post-transfection exosomes were purified from supernatants using Exo-spin.Microparticle size and distribution was determined by transmission electron microscopy (TEM) and Nanosight analysis. Exosome markers Alix, TSG101 and CD63 were detected by Western blotting. Tumorigenic properties such as invasion and anchorage-independent growth were analyzed. **Results** Nanosight and TEM revealed exosomes ranging from 50-120 nm in size. Exosomes released from ASO-T-treated cells contained the exosome markers described above, with some differences in their expression. Invasiveness was inhibited in cells treated with exosomes derived from ASO-T-treated cells, compared to controls. **Conclusions** These results suggest that exosomes released post-knockdown of ASncmtRNAs regulate tumorigenic properties *in vitro*, due to their content.

20) A COMPARATIVE STUDY OF BINDING SITES FOR NITRATE AND AUXIN IN NRT1.1

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Nitrogen is an essential macronutrient for plants and other living organisms. The main source of nitrogen for plants in agricultural soils is nitrate (NO-3). This ion is moved through specific nitrate transporters located in the plant cell plasma membrane. In addition to its role in metabolism, NO-3 can also act as a signal to regulate many biological processes in plants. The NITRATE TRANSPORTER 1.1 (NRT1.1) protein is a dual-affinity transporter that has been shown to act as a nitrate sensor. Recent studies have shown that NRT1.1 can also transport the phytohormone auxin. A competition mechanism between nitrate and auxin transport was postulated to explain root development in response to nitrate treatments. Using molecular modeling techniques, we intend to determine the molecular binding sites of nitrate and auxin in the Arabidopsis NRT1.1 transporter. We aim to search for residues that differentiate the functionality of NRT1.1-nitrate and NRT1.1-auxin. To achieve our goal, we performed molecular docking of auxin in the Arabidopsis NRT1.1 crystallographic structure and molecular dynamics simulations of NRT1.1-nitrate and NRT1.1-auxin complexes. Subsequent to this, computational mutagenesis studies were performed to finalize with calculations of binding free energy of residues proposed.

21) RATIONAL DISCOVERY OF BETA-2-MICROGLOBULIN AGGREGATION INHIBITORS AS NOVEL THERAPEUTIC AGENTS FOR CANCER TREATMENT

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Cancer is the main cause of death in developed countries, causing approximately 23% of deaths in Chile. As the costs of sequencing goes down, the capacity to obtain information of genetic variants implicated in cancer increases, allowing identification of new pharmacological targets for therapy development. Performing high throughput analyses of tumor exome data, our group identified a set of 829 proteins significantly mutated in cancer, of which 26 have been experimentally shown to form aggregates. Aggregation has traditionally been studied in the context of other diseases, like Alzheimer's disease, and there is vast literature about aggregation inhibitors. In cancer, however, aggregation has only recently been demonstrated to be implicated in the disease.

Among the 26 proteins significantly mutated in cancer for which there is evidence of aggregation, we focused on beta-2-microglobulin, component of the Mayor Histocompatibility Complex I, because of its implication in innate immunity, a crucial checkpoint in cancer progression. Characterization of variants of this protein by a theoretical strategy showed a structural rearrangement that connects two previously disconnected secondary structure elements in what seems the first step towards aggregation. Based on this information, we designed a high-throughput screening strategy to search for small drug-like compounds in public databases, which could restore the native conformation in mutants of the protein. We currently have over 5500 candidate molecules that are being exhaustively tested and our aim is to prioritize three lead compounds for experimental validation.

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22) CAP-INDEPENDENT TRANSLATION INITIATION DRIVEN BY THE HIV-1, HTLV-1, AND MMTV MRNAS IS DEPENDENT ON HYPUSINE-EIF5A.

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Replication of the human immunodeficiency virus type 1 (HIV-1) is dependent on eIF5A hypusination. EIF5A is the sole cellular protein containing the amino acid residue hypusine [*Nε*-(4-amino-2-hydroxybutyl)lysine]. Hypusine is formed post-translationally on the eIF5A precursor by two consecutive enzymatic steps; a reversible reaction involving the enzyme deoxyhypusine synthase (DHS) and an irreversible step involving the enzyme deoxyhypusine hydroxylase (DOHH). In this study we explored the effect of inhibiting DOHH activity and therefore eIF5A hypusination, on cap-independent translation initiation driven by the 5'untranslated region of the full length HIV-1 mRNA (HIV-1 IRES). We show that translation initiation mediated by the HIV-1 IRES relies on DOHH protein concentration and on its enzymatic activity. Next we evaluated the impact of eIF5A hypusination on the activity of the IRESs of the Human T-cell lymphotropic virus type 1 (HTLV-1), the Mouse Mammary Tumor Virus (MMTV), and Poliovirus (PV). Results show that eIF5A hypusination is required for the activity of retroviral IRESs (HIV-1, HTLV-1 and MMTV), but not for the activity of the PV IRES.

23) TRANSCRIPTIONAL CHARACTERIZATION OF PROTEINS RELATED TO DETOXIFICATION SYSTEMS AND DRUG RESISTANCE MECHANISMS IN *CALIGUS ROGERCRESSEYI*.

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ABSTRACT Introduction: *Caligus rogercresseyi* severely affects the salmon farming industry in Southern Chile, producing direct and indirect economic losses due to secondary infections and their respective pharmacological treatments. Currently, decreased treatments efficiencies have been reported for the drugs used to treat *C. rogercresseyi* infections. The aim of this study was to identify and characterize the expression of mRNAs coding to detoxification proteins and drug resistance mechanisms in adult *Caligus rogercresseyi* using our transcriptome databases, and structurally characterize these proteins by use of structural modeling programs. **Material and Methods**: Search and identification of sequences in the transcriptomic database. Quantitative RT-PCR was used to determine mRNA expression levels of MRP1, P-gp, CYP3A, FMO-1, UDP-GT and GST in adult Caligus. **Results**: The coding sequences for the proteins involved in detoxification mechanisms and drug resistance in our database were identified; these sequences were validated using RT-qPCR. The deduced amino acid sequences were subsequently analyzed by TMHMM and SWISS MODEL programs, for structural characterization of proteins were determined. **Discussion**: This characterization allows us to know the components involved in detoxification and drug resistance mechanisms involved in the pharmacology of *C. rogercresseyi*, which is important information for potential treatments against this parasite.

24) ATLANTIC SALMON AND COHO SALMON RESPOND DIFFERENTIALLY TO INFESTATION BY *CALIGUS ROGERCRESSEYI*

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Caligus rogercresseyi is the most problematic parasite of salmonids in seawater in Chile. The molecular mechanisms implicated in resistance/susceptibility among salmon species are scarcely understood. The aim of this study was to investigate the transcriptional responses of immune markers in the muscle/skin, anterior salmon and spleen of susceptible (*Salmo salar*) and resistant (*Oncorhynchus kisutch*) salmonids to *C. rogercresseyi* infestation. Salmonids, 72 specimens of each, were infested with 100 copepods/fish of *C. rogercresseyi* under controlled conditions. The mRNA expression levels of IL-1β, IL-6, IL-10, IL-12 and TNFα in skin/muscle, anterior kidney and spleen, of both salmonids, were studied in three different infestation periods, from copepodids (1 dpi), chalimus (11 dpi) and adult caligus (19 dpi). For skin/muscle samples, a differential response for TNFα, in both salmon species was early detected. At 11 dpi (chalimus), slight differences were detected for IL-12. The most marked differences were observed for adult caligus. Differential immune responses were also detected between healthy and damaged muscle/skin, anterior kidney and spleen for both species. For anterior kidney and spleen, the principal transcriptional changes were observed as positive modulations of most immunologic markers of *O. kisutch*, TNFα, IL-6 and IL-10 in anterior kidney. Differences in expression periods between salmonids were observed for IL-1β, TNFα and IL-10 in spleen. The increased immunological response to systemic level of *O. kisutch*, namely IL-6 of anterior kidney, IL-1β of spleen and TNFα and IL-10 of both tissues, seem to account for the lower sensitivity of this salmonid to infestation by *C. rogercresseyi.*

25) NEURONAL THY-1 INDUCED FOCAL ADHESION DISASSEMBLY IN ASTROCYTES REQUIRES PAR3 AND SYNDECAN-4.

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Thy-1 is a small glycoprotein expressed in the neuronal plasma membrane. We have previously shown that astrocyte adhesion, polarization and migration are induced by the interaction of Thy-1 in neurons with avb3 integrin and syndecan-4 in astrocytes. However, the signaling mechanisms triggered downstream of syndecan-4 remain unknown. In addition, Par3, an important adaptor protein that typically forms a complex with Par6/aPKC to induce cell polarization, reportedly, does not participate in astrocyte polarization. Therefore, we studied the participation of both syndecan-4 and Par3 in astrocyte adhesion/migration induced by Thy-1.Rat DITNC-1 astrocytes were transfected with siRNA against Par3, syndecan-4 or siRNA control, treated with Thy-1-Fc or an Fc control protein and then followed in wound-healing assays or video microscopy to monitor focal adhesion assembly/disassembly. Focal adhesion disassembly was also studied by pre-treating cells for 4 h with nocodazol. After drug washout, cells were stimulated for different time periods. We found that Thy-1 accelerated the disassembly of nocodazol-induced focal adhesions. Additionally, DITNC-1 cells lacking Par3 or syndecan-4 and stimulated with Thy-1 migrated less and the disassembly of their focal adhesions was delayed compared to control cells. Moreover, focal adhesions were larger in cells lacking Par3, but smaller in syndecan-4-deficient cells than in control cells, implicating both proteins in astrocyte adhesion induced by Thy-1. These results suggest the existence of a hitherto unexplored role for Par3 and syndecan-4 in the regulation of focal adhesion turnover.

26) CHARACTERIZATION OF LIGAND BINDING BETWEEN P2X2 RECEPTOR AND γ-[2-AZIDOETHYL]-ATP ANALOGUE BY SINGLE MOLECULE ATOMIC FORCE SPECTROSCOPY AND PATCH-CLAMP

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P2X2 receptor is a ligand-gated ion channel expressed throughout the nervous system, involved in cancer, nociception and enteric neurotransmission where expression levels of both the receptor and its ligand ATP remain unbalanced. At molecular level, it is essential to understand the interaction between ATP and P2X2, from their binding up to the ion current through the channel. Accordingly, individual P2X2 receptors were studied through its interaction with an ATP analogue functionalized in AFM tips. Firstly, purified P2X2-His6 receptor was inserted onto liposomes, obtaining 6nm height for receptor ectodomain, consistent with its crystal structure. Secondly, rupture of the ligand-receptor complex was characterized by single molecule force spectroscopy, obtaining kinetic and thermodynamic parameters that define it as low force long-lasting interaction, which occurs spontaneously but must overcome a huge energy barrier to succeed. Thirdly, using patch-clamp recordings cells expressing P2X2 receptor were used to measure the Na-current in response to agonists. ATP had greater efficacy and potency than its analogue, which did not desensitize the receptor, suggesting that γ-[2-Azidoethyl]-ATP is a partial agonist of P2X2, adopting different 3D configuration at the binding pocket that results in bond loss with R290 residue. Coupling both binding and current results, the energy required for the receptor to make conformational rearrangements from closed to open states was estimated in 50,32 kJ/mol. These results give structural insights for the association between the receptor-ligand binding process and the functional ion current at physiological environment. Acknowledgments Fondecyt 1120169, DPI Newton Picarte Conicyt 20140080.

27) STABILITY OF ECE-1C AND ITS ROLE IN PROLIFERATION AND MIGRATION ARE ENHANCED BY PROTEIN KINASE CK2 IN COLON CANCER CELLS.

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Introduction. CK2 is a serine/threonine protein kinase overexpressed in several types of cancer. Over 300 of phosphorylatable targets have been reported, most of them relevant in cancer. ECE-1c is a metalloprotease, which may act as a mitogen to promote cancer progression. We have previously demonstrated that the N-terminal of ECE-1c is phosphorylated by CK2 at serines 18 and 20. In this work, we evaluated whether the stability of a non-ubiquitinable ECE-1c was affected under pharmacological inhibition of CK2, as well as its role in proliferation and migration in colon cancer cells. Methodology. Three ECE-1c mutants were developed and cloned in a mCherry-encoded lentiviral plasmid: ECE-1c-K6R (K6R: non-ubiquitinable), ECE-1c-S18A-S20A (AA: non-phosphorylatable) and ECE-1c-S18D-S20D (DD: CK2-phosphomimetic). ECE-1c mutant transfection efficiencies were evaluated through the bicistronic expression of mCherry. Stability assays were performed in murine CHO-K1 cells using CHX, and CK2 inhibition was achieved using CX-4945. Cell proliferation and migration assays were conducted in DLD-1 colon cancer cells transfected with the ECE-1c mutants using Flow Citometry and Transwell, respectively. Results. A 70% transfection efficiency was obtained for ECE-1c mutants on the murine CHO-K1 cells. CHX assays showed higher stability of K6R and DD mutants in the presence of CX-4945, while AA's stability was lower than wild-type. Cell proliferation and migration showed to be higher in the DD mutant. Conclusions. Phosphorylation of ECE-1c on the residues S18 y S20 by CK2 promotes its stability, which enhances proliferation and migration of colon cancer cells.

28) MILD-TRAUMATIC BRAIN INJURY ALTERS NMDA RECEPTOR DISTRIBUTION AND ASSOCIATED SIGNALING

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Introduction: Traumatic Brain injury (TBI) mediate neuronal death through a series of events that involve several molecular pathways as glutamate-mediated excitotoxicity for excesive stimulation of N-metyl-D-aspartate receptors (NMDARs) producing free-radical generation and asociated death pathways. However changes in NMDARs (distribution and signaling-asociated to the distribution) remain incompletely understood. **Methodology:** TBI was performed by a controlled frontal impact device, cognitive performance was evaluated by Morris water maze task, alterations in synaptic transmission was evaluated by field evokated excitatory postsynaptic potentials and the levels of phospho-CREB, STEP and oxidative stress markers was evaluated by immunofluorescence. **Results and conclusions:** After TBI, we found alterations in cognitive performance in mice and synaptic function NMDAR-dependent, increase in oxidative stress markers and changes in molecular targets implicated in the NMDARs distribution as an increase of STEP, phosphatase which decreases the synaptic form of NMDARs and decrease of pospho-CREB, transcriptional factor activated by synaptic response. Using a model of mild TBI in mice, we provide a mechanistic understanding of the signaling pathways compromised in the excessive presence of ROS resulting in an alteration of NMDARs, and the balance between synaptic and extrasynaptic signaling, with consequences in brain functions, possibly by controlling NMDARs trafficking and delivery to the plasma membrane.

29) UNUSUAL DIMERIZATION OF A *BC*CSP MUTANT *VIA* ITS NUCLEIC ACID-BINDING SURFACE LEADS TO ALLOSTERIC CONFORMATIONAL DYNAMICS

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Cold shock proteins (Csp) constitute a family of ubiquitous small proteins, whose function is to act as RNA-chaperones to avoid cold-induced termination of translation. All members of this family contain two subdomains connected by a hinge loop, composed by 2 and 3 β-strands respectively. Both subdomains fold into a compact β-barrel that has been usually characterized to exist as a monomer, although there are a few examples that show a higher aggregation state. We focused on *Bacillus caldolyticus* Csp (*Bc*Csp), whose folding, function and structure have been largely studied. Although *Bc*Csp has been described as a monomer following a two-state folding mechanism, a crystal structure showed dimerization via domain swapping (DS). Consequently, we previously engineered *Bc*Csp to allow DS in solution by deleting two residues from the hinge loop (*Bc*CspΔ36-37), leading to a protein in monomer-dimer equilibrium without stability changes compared to wild-type *Bc*Csp. However, the crystal structure of *Bc*CspΔ36-37 dimer showed that its interface is the ligand-binding surface, similarly to a dimer of CspE from *Salmonella typhimurium*, rather than being formed by DS. Here we demonstrate, by circular dichroism, that ligand binding disrupts the *Bc*CspΔ36-37 dimer, confirming the conformation seen in the crystal structure. Moreover, structure-based molecular dynamics and hydrogen-deuterium exchange mass spectrometry show that, while the *Bc*CspΔ36-37 monomer is as flexible as the wild-type *Bc*Csp, the overall conformational dynamics are severely reduced upon dimerization. Our results reveal an allosteric effect triggered by dimerization and enlighten the interplay between conformational dynamics and oligomer evolution.

30) HYPOXIA INDUCED CAVEOLIN-1 EXPRESSION PROMOTES MIGRATION OF CANCER CELLS

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Introduction: Exacerbated proliferation of cancer cells in nascent tumors leads to nutrient deprivation and the genesis of an hypoxic microenvironment. Hypoxia is associated with poor patient prognosis because this stress condition selects for more aggressive tumor cells with enhanced migratory, invasive and metastatic capacities. These changes are associated with the induction of the hypoxia-induced factor (HIF) and increased expression of target genes, including the scaffolding protein caveolin-1 (CAV1). Results from our group have shown that CAV1 expression in metastatic cancer cells promotes cell migration/invasion *in vitro* and metastasis *in vivo.* Here, we evaluated whether hypoxia-induced expression of CAV1 was required to promote hypoxia-dependent migration of cancer cells. **Methods:** B16F10 murine melanoma and HT29(US) colon adenocarcinoma cells were exposed to hypoxia (1% O_2) for 24 h. Induction of CAV1 expression was evaluated by western blotting. HIF transcriptional activity was evaluated in reporter assays. Endogenous CAV1 was knocked-down using specific shRNA constructs. Cell migration was evaluated in Boyden Chamber assays. **Results:** Hypoxia induced HIF-dependent transcription and augmented CAV1 protein levels in B16F10 and HT29(US) cells. Importantly, hypoxia promoted migration of both tumor cell models in a CAV1dependent manner, since CAV1 knockdown reduced hypoxia-induced migration. **Conclusions:** Hypoxia induced migration of metastatic cancer cells requires induction of CAV1.

31) THERMAL STABILITY OF HUMAN ADENOSINE KINASE

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Adenosine kinase (AdeK) is a monomeric enzyme responsible for converting intracellular adenosine to adenosine monophosphate (AMP) using ATP as phosphoryl donor. The enzyme presents low stability at low concentrations and its catalytic activity is increased in the presence of osmolytes, although the mechanism of this activation is unknown. Adek is member of the ribokinase family whose members are mainly dimers, such as phosphofructokinase-2(Pfk-2) from *E. coli*, which unfolds by a three state mechanism [$N_2$2I2U], where the dissociation of the dimer is highly cooperative while the monomeric intermediary shows low unfolding cooperativity. A monomeric mutant of Pfk-2 (L93A) unfolds noncooperatively. At present, the stability and unfolding of monomeric enzymes of this family has not been evaluated. In this work we have studied the stability and thermal unfolding of AdeK, evaluating the effect of ionic strength and betaine as osmolyte using circular dichroism . The results show a low cooperativity unfolding at low ionic strengths upon increasing temperature, while under high ionic strength or betaine the unfolding is highly cooperative. At low ionic strength AdeK has a lower content of helical structure compared to high ionic strengths or the presence of betaine. According to these results the low stability and low catalytic activity of AdeK may be due to its partial unfolding under conditions of low ionic strength or low osmotic pressure.

32) STRUCTURAL ANALYSIS OF ADP-DEPENDENT KINASES FROM HALOPHILIC ORGANISMS OF THE ORDER *METHANOSARCINALES*

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Proteins from halophilic organism are able to carry out their function in the presence of molar concentrations of salt. Most of the work aimed to understand the structural adaptations of these proteins has been done using proteins from the archeon class *Halobacteria*. Proteins from these organisms are characterized by a low abundance of basic residues and a high amount of acidic residues, which accumulate in the protein surface. Nevertheless, halophilic organisms have been reported in a wide variety of taxa, including other archeons orders and also key signatures of structural adaptations to hypersaline environments are not yet established. To evaluate the ubiquity of these mechanisms of adaptation, we built homology models of ADP-dependent kinases from halophilic and non-halophilic organism of the archaeal order *Methanosarcinales* and compared them with models from the *Natrialbales* order (*Halobacteria)* and eukaryotic ADP-dependent kinases. We analyzed the distribution of residues between the proteins surface and core. Our results show that proteins from the order *Methanosarcinales* do not exhibit the classical adaptations present in Halobacterial proteins, like negative charge in the protein surface and reduction of the hydrophobic core. However, both groups have a similar amount of long range ionic interactions. These results suggest that the adaptations required for maintaining the solubility and function of a protein in high salinity environments can vary widely between non related organisms.

33) EFFECT OF METHYL-JASMONATE ON FLAVONOID ACCUMULATION IN ANTARCTIC *COLOBANTHUS QUITENSIS* (KUNTH) BARTL.

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C. quitensis is the only dicot that naturally inhabits in Maritime Antarctica, in this place, physicochemical components of the environment generate constantly a selective pressure. In literature, some biochemical characteristics of *C. quitensis* have been described as an important factor that mediate its capacity to survive, in this context, phenolic compounds represents an important milestone, to mediate for example the tolerance to abiotic factors. The aim of this work is evaluate the effect of methyl-jasmonate (MeJa) as an inductor of phenolic compounds in *C. quitensis*. Our results show an induction in the key enzyme of phenylpropanoid pathway, phenylalanine ammonia-lyase (PAL), and flavone *C*-glycosides metabolites, from 10 to 50 µM of MeJa, and repression at high concentrations (100-500 µM). In conclusion, MeJa is a good candidate to elicit accumulation of flavonoinds in *C. quitensis* with biotechnological prospection.

34) CARACTERIZATION OF A RETROTRANSPOSON IN *MYTILUS CHILENSIS.*

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Haemic neoplasia (HN) is a proliferative cell disorder of the circulatory system of bivalve mollusks. Elucidation of the etiology of HN has been a key issue since the discovery of the disorder (at least for one specie; the soft shell clam Mya arenaria) is a transmissible cancer. The disease is transmitted between individuals and can also be induces by external agents such as bromodeoxiuridine in M. arenaria. In M arenaria, we have cloned and characterized a retrotransposon named Steamer. Steamer mRNA levels and copy number correlates with disease status and can be used as a marker of the disease.

It has been described that Chilean mollusks, particularly Mytilus chilensis (chorito) can suffer HN. We wonder if there was a retrotransposon with similar characteristics to Steamer in M chilensis.

We interrogate a preliminary reference transcriptome of *M chilensis* with the full length M arenaria Steamer DNA sequence. We found one contig of 4545 nucleotides where 3515 nucleotides align to Steamer with a 64% identity. The contig encodes for one open reading frame (ORF) of 1376 aminoacids with conserved domains for reverse transcriptase, RNAseH, protease and integrase domains, strongly suggesting of a retrotransposon. Interestingly the ORF also contain a PKC domain. Currently, we are cloning the putative retrotransposon from the genome of *M chilensis*, and through genome walking strategy, we expect to find LTRs and the integration site. A possible role of this new retrotransposon as a marker of HN in M chilensis will be determined in the future.

35) CHARACTERIZATION OF STRESS-ASSOCIATED MICRORNAS IN *PARALICHTHYS ADSPERSUS* SKELETAL MUSCLE USING SMALL-RNA DEEP SEQUENCING

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Introduction: MicroRNAs are ubiquitous, noncoding, short RNA molecules playing important functions as post-transcriptional regulators of gene expression. Previous studies characterized the transcriptomic response associated to confinement-stress stimulus in the skeletal muscle of the Chilean flounder *Paralichthys adspersus*, an endemic species with great aquaculture potential. However, until now there was no information available on the involvement of microRNAs in confinement-stress response.

Materials and Methods: RNA from skeletal muscle of confinement-stressed and unstressed *P. adspersus* was collected and sequenced. The small RNA sequences identified by Illumina small RNA deep sequencing were blasted against miRBase database v.21. The differential expression analysis of microRNAs between stressed and unstressed samples taken at 4 and 7 weeks (4w and 7w) was performed using CLC Genomics Workbench software. In addition, the 3´UTR sequences of several genes known to be involved in muscle growth and stress were compiled and two software tools (PITA and miRanda) were used to predict target genes of the differentially expressed microRNAs.

Results: We obtained 39,405,058; 36,351,318; 33,605,168; and 37,315,402 high-quality, filtered reads for 4w-stressed, 4w-unstressed, 7w-stressed and 7w-unstressed muscle samples, respectively.Considering all libraries, 334 known mature microRNAs were identified, with a total of 19 known microRNAs exhibiting significant differential expression (Fold change ≥|4|; $p<0.05$; and normalized expression > 3) between libraries. *In silico* target prediction suggested putative roles in the stress response of *P. adspersus* muscle.

Discussion: These differentially-expressed microRNAs could exert novel regulatory functions in *P. adspersus* muscle, and might be useful as biomarker candidates for stress response in other teleosts.

36) PROSTATIC ACID PHOSPHATASE PROMOTES EPITHELIAL-MESENCHYMAL TRANSITION AND CELL MIGRATION/INVASION IN GLIOBLASTOMA STEM-LIKE CELLS THROUGH LOW-AFFINITY ADENOSINE RECEPTORS ACTIVATION UNDER HYPOXIA

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Introduction: Glioblastoma multiforme (GBM) is one of the most lethal brain tumours, highlighting their invasive ability to infiltrate healthy tissue leading to recurrence. A cell subpopulation, Glioblastoma Stem-lik eCells (GSCs) has been reported as responsible of these invasiveness properties. Inside the hypoxic tumour niche promotes Epithelial-MesenchymalTransition (EMT), acritical step for migration/invasion process. Moreover, hypoxia increases extracellular Adenosine levels, which activates the Low-Affinity Adenosine Receptors (L-AARs) A_{2B}AR and A_3AR. We postulate that high concentration of Adenosine under hypoxia is mediated by ectonucleatidases (CD73 and/orPAP) in GSCs, which promotes EMT and cell migration/invasion through L-AARs activation. **M&M:** GSCs of U87 GBM celll in were cultured under normoxia and hypoxia. Ectonucleotidases, L-AARs and EMT markers expression were evaluated by RT-qPCR and westernblot. AMPase activity was measured by HPLC. Cell migration was measured by wound-healing and neurosphere attachment assays. Invasion was evaluated by agarose spot and transwell matrigel-coatedassays. To determine the ectonucleotidase activity and its effect on cell migration/invasion, CD73 and/or PAP knockdown celll in es were generated. The contribution of LAAR son cell migration/invasion and expression of EMT markers were evaluated by selective A_{2B}AR and A_3AR antagonists. **Results:** Cell migration/invasion and ectonucleotidase activity of GSCs were higher under hypoxia than normoxia. Knockdownof CD73 and PAP decreased cell migration/invasion under normoxia and hypoxia, respectively. Blockage of L-AARs decreased cell migration/invasion and EMT markers. **Conclusion:** High extracellular Adenosine concentration generated by increased activity of PAP under hypoxia enhances cell migration/invasion in GSCs, increasing EMT markers expression through L-AARs activation.

37) MISFOLDED NEWLY SYNTHESIZED HISTONE H3 IS DEGRADED BY THE UBIQUITIN-PROTEASOME SYSTEM

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INTRODUCTION: To acquire its correct conformation and characteristic post-translational modifications, the newly synthesizedhistone H3 is associated, in a sequential manner, with different enzymes and chaperones through four different cytosolic complexes in a “cascade of maturation”. The soluble histone levels are highly regulated in the cell because of its potential harmful effect, so that prompted us to investigate the degradation mechanisms involved in the response to cytosolic misfolded histone H3.

METHODOLOGY: Protein misfolding was induced in HeLa cells by treating them with the amino acid analogues *L*-Azetidine-2-carboxylic acid (AZC), and L-Canavanine (Can). To evaluate the participation of the ubiquitin-proteasome system (UPS) in the degradation of misfolded histone H3, the cells were exposed to the proteasome inhibitor MG132, together with the analogues. Then, cytosolic extracts were obtained and analyzed by Western-blot to asset the variations in the levels of the different chaperones and the histone H3.

RESULTS: We found that using both, MG132 and amino acid analogues, an accumulation of cytosolic histone H3, greater than the generated with the drugs separately, occurred.

CONCLUSIONS: Our results suggest that the ubiquitin-proteasome system (UPS) has a role in the degradation of the misfolded newly synthesized histone H3.

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38) KNOCKDOWN OF ANTISENSE NON-CODING MITOCHONDRIAL RNAS UPREGULATES MIRNAS INVOLVED IN CELL CYCLE REGULATION IN HUMAN BREAST CANCER CELLS

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The family of non-coding mitochondrial RNAs (ncmtRNAs), comprising Sense (SncmtRNA) and Antisense (ASncmtRNA) members, displays differential expression between cancer and normal cells has been studied in our laboratory as a tool to develop a selective cancer therapeutic approach. Targeting of ASncmtRNAs with chemically modified antisense oligonucleotides (ASO), 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 downregulation of Cyclins D1 and B1. We performed profiling of small RNAs of the human breast cancer cell line MDA-MB-231 by next generation sequencing. Knockdown of ASncmtRNAs in this cell line shows upregulation of several miRNAs having either validated of predicted targets in Cell Cycle KEGG pathway. In addition, we discovered novel miRNAs coded in the nuclear and/or mitochondrial DNA. Interestingly, miR-4485-3p and miR-1973 are found expressed in the same transcript in several reads, which map to the mitochondrial DNA, but not to the published origin of these transcripts in two different chromosomes. This work provides evidence of the role of ncmtRNAs on regulation of cell proliferation by altering miRNA levels and as potential precursors of mito-miRNAs previously annotated as nuclear.

39) EFFECT OF GLUCOCORTICOID-INDUCED STRESS ON THE GROWTH PATHWAY AND MUSCLE ATROPHY IN THE FINE FLOUNDER (*PARALICHTHYS ADSPERSUS*)

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Introduction: The chronic stress is one of the main factors, which negatively impacts growth in fish. The response to stress increases glucocorticoid (cortisol) secretion, mediated through the hypothalamic-pituitary-interrenal axis. An important tissue for growth regulation is skeletal muscle, which is fundamental for metabolic and physiological processes. In mammals, it is well documented that under stressful conditions increased glucocorticoid levels are associated to skeletal muscle atrophy, however relatively little is known about this condition in teleost. The aim of this study was to evaluate the effect of dexamethasone (DEX), a synthetic GC, on the growth pathway and muscle atrophy in the fine flounder.

Materials and Methods: Juvenile fish were used, allocated into 4 groups of 4 individuals each. To stimulate glucocorticoid-induced stress, one group was injected with DEX, another group with RU486 (a GR blocker) and another with both drugs. Control groups were injected with the vehicle (EtOH). After 72 hours of treatment, skeletal muscle was collected and stress-related genes (*gr1, gr2, mr, klf15 and redd1*) were evaluated by RT-qPCR or Western blot.

Results: No significant changes were observed in the transcript levels of the genes assessed after the DEX treatment. However, major changes were observed after blocking the GC receptors with RU486. Here, *mr, klf15* and *murf-1*mRNA levels were increased. Furthermore, AKT activation and IGF-1 levels were unaffected in all the treatments.

Conclusion: These results suggest that the inactivation of GC receptor, the ligand/receptor balance could be shifted to mineralocorticoid pathway, promoting muscle atrophy through the ubiquitin-proteasome system.

40) HMMTEACHER1.0 : A TEACHING RESOURCE FOR UNDERSTANDING HIDDEN MARKOV MODEL SOLUTIONS THROUGH PRACTICE.

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Hidden Markov Models (HMM) are one of the most powerful and obscure modeling techniques in Bioinformatic Sequence Analysis, in spite of - or maybe caused by - being theoretically well supported. Other applications of HMMs include speech recognition and topics in structural biology. Their use has increased in time in a shy way, in part because: (1) its steep theory learning curve; (2) the need of good modeling and programming skills in order to implement HMM algorithms and;(3) because HMMs have to be fed with large amounts of carefully curated data. In order to facilitate the use, understanding and practice of HMMs, we have developed a Java based software that solves HMM algorithms once the user inputs the model.The graphical interface guides the learner through five steps: Steps 1 to 3, requesting the main elements of a HMM; 4.Asking the user which one of the three questions (related to algorithms Forward, Backward and Viterbi) is to be answered and; 5.Reporting the solutions step-by-step in PDF format document. In the case the user doesn't know the information the software is requesting, it provides an option in which the program fills the unknown box with random generated numbers. This way, the student can go on and learn by examining the solution of the model. This first version allows the Bioinformatics teacher to focus the HMM classes on modeling the biology and in the problem solving practice instead of on the mathematical theory that leads to the algorithm formulae.

41) UNDERSTANDING THE BINDING DETERMINANTS OF VERATRYL ALCOHOL AT SURFACE OF LIGNIN PEROXIDASE FROM *P. CHRYSOSPORIUM*: A MOLECULAR DYNAMICS AND MM-GBSA BASED STUDY

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Lignin peroxidase (LiP), a fungal heme-containing peroxidase first discovered in the basidiomycete *Phanerochaete chrysosporium*, plays an important role in the degradation of lignin and lignin model compoundsdue to its high redox potential.

Veratryl alcohol (VA), is a secondary metabolite of the fungus *P. chrysosporium* and is the main substrate of LiP. Also, VA acts as a redox mediator after being oxidized to a radical species ($VA^{\bullet+}$) by Trp171, a catalytic residue located at the protein surface.

In a previous report, we have explored through molecular docking, MD and MM-GBSA simulations the way VA (in its neutral state) interacted with Trp171 and how it was stabilized by other residues at the protein surface.

Furthermore, VA in a neutral and cationic state was used to run long molecular dynamics of 1μs for LiP-$VA^{\bullet+}$ and LiP-VA complexes with Desmond software. Interaction profiles for each state of VA were obtained showing that there exists a clear difference in the interaction dynamics for both species. For a further understanding of the stabilization mechanism of VA species at the protein surface, in this work are reported new long MD simulations that take into account the appropriate substrate concentration and explores its affinity by WT LiP and E168Q and D264N mutants. Computational results are compared with in house binding affinity experiments of LiP, and the abovementioned mutants, expressed in *P. pastoris*.

42) ATOMISTIC STRETCHING AT EXPERIMENTAL PULLING SPEEDS THROUGH STRUCTURE-BASED COMPUTATIONAL TWEEZERS

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Constant-velocity pulling experiments are the most widespread force spectroscopy techniques to assess the energetics of folding, binding and activity for different proteins or biomolecular ensembles. Although there are a growing number of proteins whose mechanical behavior is well understood, they represent a minimal fraction when compared to the huge number of protein structures available. Therefore, several molecular dynamics approaches have sought to correlate structural data with experimental mechanical properties, but they normally rely on either extremely high pulling speeds, or greatly reduced system details.

In this work we employed atomistic structure-based models (SBM), based on the energy landscape theory of protein folding, to explore the computational constant-velocity stretching of 31 experimentally well-studied, mechanic-benchmark proteins ranging 10-64 kDa. Using a previously reported time-step calibration of atomistic SBMs, we were able to reproduce the stretching of all proteins at experimental pulling rates and to correlate the experimental and simulated force rip values. Such force calibration gave us a slope of 0.70 and R^2 of 0.84, therefore every reduced force unit in these models represents 1.43 pN. While this model agrees with previous computational strategies using coarse-grained SBMs, it enables detection of intermediates unseen before, as in the case of titin, which we argue is mostly due to the explicit representation of side chains. We further tested this model on the transformer protein RfaH, obtaining a mechanical unfolding pathway consistent with previous reports. Our results put forward a computationally efficient strategy to resolve atomistic details of the mechanical resistance of proteins.

43) PREDICTING THE EFFECT OF P294A VARIANT ON MICA PROTEIN IN GASTRIC ADENOCARCINOMA.

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Gastric cancer (GC) is the principal causes of cancer mortality in Chile. Its current incidence is 30 per 100,000 habitants. MHC class I chain-related gene A (MICA) acts as a ligand for natural killer(NK) cells and other cytolytic cells bearing the NKG2D receptor. Several studies indicate that single-nucleotide variant (SNV) of the MICA gene have been associated with tumor immune evasion. Here we investigated the presence of somatic mutations in MICA gene by targeted sequencing in GC patients. Genomic DNA was isolated from tumor tissue samples of 50 patients with GC who underwent gastrectomy. Targeted sequencing with Illumina MiSeq TruSeq Custom Amplicon (TSCA; 12 amplicons, 500x coverage) was then carried out. The bioinformatic analysis of sequences was performed with the algorithm Somatic Variant Caller(Illumina). All research was performed in agreement with institutional ethical committees. Three new variations were detected, this had not been previously described in databases, and according to SIFT-algorithm only the variant chr6:31380116 would have an effect on MICA protein. Also, were identified 6 SNV somatic mutations according to allele frequencies. In relation to the data released by the database SNPeffect4.0 only variant P294A presented a reduction in the MICA protein stability. This change is located in a region of possible proteolytic cuts by metalloproteases, and could affect the release of ligands by the tumor cell. Our results suggest that the presence of SNVs in the MICA gene it might be related with immune evasion strategy which provides establishment and development of tumor.

44) BLOCKADE OF ADENOSINE A_3 RECEPTOR PREVENTS IL-1β AND IL-18 PRODUCTION AND RENAL FIBROSIS IN DIABETIC RATS.

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Introduction. Diabetic nephropathy (DN) is the leading cause of end-stage renal failure whose pathogenesis must to be elucidated. The progression of DN has been associated with a progressive and irreversible renal fibrosis and elevated levels of adenosine. Adenosine signaling through the A_3 receptor promotes mesenchymal transition of tubule epithelial cells in vitro. Our objective was to evaluate the effects of an adenosine A_3 receptor antagonist on renal fibrosis in diabetic rats.

Methods. Experimental diabetes was induced in rats by using streptozotocin (STZ). Rats were treated with the adenosine A_3 receptor antagonist MRS1220 at 0.1 mg/kg i.p. for 1 month. The kidneys were extracted for immunohistochemical analyses of fibrosis markers. Urine was collected for measuring of inflammatory cytokines by Immunoassays & MILLIPLEX® map system.

Results. Renal fibrosis in diabetic rats was blocked by using the adenosine A_3 receptor antagonist MRS1220, decreasing the expression levels of the fibrotic marker α-SMA. Also, proteinuria was decreased in MRS1220 treated rats. In vivo, the use of the A_3 receptor antagonist inhibited production of inflammatory IL-1β and IL-18 in diabetic rats while changes in IL-6, IL-10, TNF-α and MCP-1 levels were not observed.

Conclusions. The renal fibrosis triggered by inflammatory mediators IL-1β and IL-18 can be blocked by a pharmacological A_3 receptor antagonist in diabetic rats, which supports the involvement of adenosine signaling as a new therapeutic alternative.

45) GDF11 EXERTS ANTI-HYPERTROPHIC AND METABOLIC ACTIONS ON CULTURED CARDIAC MYOCYTES

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Cardiac Hypertrophy is a adaptative mechanism in response to mechanic and neurohumoral stimuli that is characterized by increase in the cardiomyocyte and heart size associated to increase in the contractile protein levels and the sarcomeres numbers. However, the chronic exposition to stressors lead a pathologic phenotype with loss of contractile function. Our previous works showed that the hypertrophy cardiomyocyte decrease the mitochondrial energetic metabolism and ATP levels, and its maintenance are esencial for the contractile function of cardiomyocyte. Growth/ Differentiation Factor 11 (GDF11), belonging to TGF-β superfamily, has showed to prevent *in vitro* the cariomiocyte hypertrophy norepinephrine induced. Our goal is search to mechanism that GDF11 produce this effect on hypertrophy cardiomyocyte. For that, we used neonatal rat cardiomiocytes treated with pro-hypertrophic stimulus norepinephrine (NE, 10 uM) and evaluated the effects of recombinant human GDF11 (rGDF11). Results showed rGDF11 is able to induce the phosphorylation of Smad2 in a concentration-dependent manner, obtaining the maximum response to 10 nM. rGDF11 prevented the increase cardiomyocyte area and ßMHC and pro-ANP-induced proteins NE, evaluated by microscopy using rhodamine-phalloidin the probe and Western blot, respectively. By qPCR was observed that GDF11 did not prevent the increase ANP and BNP mRNA, indicating that perhaps the effect are not gene. Addicionality, rGDF11 was able to prevent the decreased of ATP levels induced by NE. Collectively these results suggest that GDF11 prevents hypertrophy induced by NE through its action in the energy metabolism.

46) CHARACTERIZATION OF THE SUBCELLULAR LOCALIZATION OF THE PROTEIN X (HBX) ISOFORMS OF HEPATITIS B VIRUS (HBV)

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The X protein (HBx) of the hepatitis B virus, is a 154-amino acid non-structural protein without structural homology with other known proteins. HBx is key in the regulation of viral infection, replication and possibly carcinogenesis. HBx gene has three ATG codons in reading frame, generating three HBx isoforms, a full length protein of 17 kDa, a medium protein of 8 kDa and a small protein of 6.6 kDa. It has been reported that these isoforms individually are able to activate class III promoters, while together they can activate class II promoters. The subcellular localization of HBx is dependent on its expression levels, located mainly in the cytoplasm at high expression levels or nuclear at low expression levels. In this work, in order to study the location of each isoform separately when varying the expression levels of each HBx isoform tagged them with either GFP or 3xFLAG and performed fluorescence and immunofluorescence. We found that the full length protein is localized as reported, while the median protein has the opposite localization. When studying the location of these two proteins together, the full length protein is relocated into the nucleus. We concluded that each isoform is localized differently, but they can modulate their location when expressed together.

47) γCDCPLI, A PLA_2 INHIBITOR FROM *CROTALUS DURISSUS COLLILINEATUS* SERUM, INDUCES APOPTOSIS ON BREAST CANCER BY PI3K/AKT AND P53 PATHWAY MODULATION

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Endogenous phospholipase A_2 (PLA_2) has been currently investigated by its capacity to induce cell growth, survival and invasion of tumor cells. In this context, we explored the antitumor effect of PLA_2 inhibitor, named γCdcPLI, isolated from *Crotalus durissus collilinetatus* serum in MDA-MB-231 human breast cancer cell line. In this work, the cytotoxicity of γCdcPLI was evaluated by MTT technique and apoptosis was analyzed by Annexin V/Propidium Iodide. The levels of prostaglandin (PGE2) and leukotriene (LTB4) at culture supernatants were quantified by commercial enzyme immunoassay kit. In addition we evaluated gene expression of MDA-MB-231 cells by RT/PCR (Real Time PCR) and the protein expression by Western Blotting. γCdcPLI was cytotoxic to MDA-MB-231 cancer cells (IC_{50} of 25µg/mL) and interestingly, it did not affect the viability of control non-tumorigenic breast cell MCF10-A. The late apoptosis of MDA-MB-231 was observed after 24 h treatment with γCdcPLI at 25µg/mL and 50µg/mL. In addition, γCdcPLI treatment was able to decrease significantly PGE2 level. RT/PCRanalysis demonstrated an up- and down-regulation of different genes related to apoptosis and survival pathway, and the RT/PCR data were confirmed by Western blotting. Taken together, these data indicate that γCdcPLI induces antitumoral action by decrease PGE2 level, PI3K/AKT pathway inhibition and overexpression of p53 pathway. Thus, γCdcPLI showed a high potential to understanding the mechanism of growth and survival of breast cancer cells. We hope this study will contribute to investigating the potential use of these inhibitors with models of cancer treatment.

48) ANTI-ANGIOGENIC ACTIVITY OF PLA2 LYS49 BNSP-7 ISOLATED FROM BOTHROPS PAULOENSIS VENOM

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Tumorigenesis occurs because of the unbalance between pro and anti-apoptotic factors which promotes cell survival, proliferation and growth of cells that carry mutations. However, a tumor is formed and maintained due to a combination of factors that provide support and assist its needs. Blood supply is an essential need because it supplies nutrients, new blood vessels are than formed through a process called angiogenesis. Thus, drugs that aim to inhibit angiogenesis have high therapeutic potential and biological compounds, e.g., snake venoms are target of new researches. Phospholipases A2 (PLA2) isolated from the snake venom showed anti- angiogenenic activity. This work shows the anti-angiogenic effects of BnSP-7, a Lys 49 PLA2 isolated from Bothrops pauloensis venom, on Human Umbilical Vein Endothelial Cell (HUVEC). BnSP-7 isolation was performed by two chromatographic steps by CM-Sepharose and RP-HPLC. The molecular mass and homogeneity of BnSP-7 were evaluated by 12.5% (v/v) SDS/PAGE. The HUVEC cells viability was determined by MTT assay and cell adhesion inhibition assay was performed using three different substrates, fibronectin, collagen and matrigel and analyzed through MTT assay. Inhibition of in vitro vessels formation of HUVEC cells was performed by matrigel coating assay. BnSP-7 showed to be homogeneous by SDS-PAGE and it was not cytotoxic to HUVEC cells at different concentrations. BnSP7 was also able to interfere on adhesion and vessels formation on different substrates. The BnSP-7 demonstrated anti-angiogenic activity on HUVEC, however, more experiments need to be made to confirm its pharmacological potential as an anti-angiogenic drug.

49) GENERATING A SYNTHETIC HYBRID OSCILLATOR THROUGH TRANSCRIPTIONAL REWIRING

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Circadian rhythms are present in diverse organism, from bacteria to mammals, being the molecular bases that govern them quite conserved. These rhythms are generated at the cellular level by a transcriptional-translational negative feedback loop, where the negative element inhibits the activity of the positive element that controls its expression. They are also self-sustained and in constant conditions (darkness) have periods close to 24 hours.

In *Neurospora* the negative element is the protein FRQ, encoded by the gen *frequency* (*frq*) and the positive element is the White Collar complex (WCC), composed by the transcription factors White Collar 1 (WC-1) and White Collar 2 (WC-2), where WC-1 is also a photoreceptor. The oscillator transmits the time information to various biological processes such as growth and metabolism. This is mediated, in part, by a hierarchical arrangement of transcription factors; which allow the rhythmic expression of genes controlled by the clock. To improve our knowledge of *frq* transcriptional regulation and of the plasticity of the clock we have utilized transcriptional rewiring, as part of a synthetic biology approach, to generate a new topology of the central oscillator, evaluating the ability of the system to generate and sustain rhythms. We have also challenged the system with different environmental and genetic perturbations to test its behavior. Using this new approach we demonstrated the ability of the *Neurospora* clock to sustain rhythms, even when the architecture and crucial properties of the central oscillator are severely modified, revealing an unexpected plasticity to transcriptional and environmental inputs.

50) MOLECULAR CHARACTERIZATION OF COREST-HDAC2 COMPLEX.

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Epigenetic regulation of gene expression involves the active participation of chromatin remodeling complexes. Our research group studies a co-repressor complex constituted by the histone deacetylases HDAC1/2 and the transcriptional co-repressor CoREST. The CoREST-HDAC1/2 complex participates in several biological processes including neuronal differentiation and epithelial mesenchymal transition. CoREST as other HDAC1/2 recruiting proteins, interacts with HDAC1/2 throughout its ELM2-SANT1 domains, located on its amino-terminal region, however the Molecular Mass (MW), Stoichiometry and Molecular Architecture of the complex are not known. Here we have addressed these issues by analyzing the recombinant CoREST-HDAC2 complex using Size Exclusion Chromatography (SEC) and Mass Spectrometry (ESI-MS). The elution volume (V_e) of the CoREST-HDAC2 complex points out to a MW of 150kDa, indicating that the proteins may form a tetramer. ESI-MS experiments were difficult to perform because the protein sample precipitated very fast in Ammonium Acetate, however we were able to identify a native peak around 150-160kDa, but deeper ESI-MS/MS analysis was impossible to get. In order to better determine the MW and Molecular Architecture of the complex we will perform direct imaging of the recombinant CoREST-HDAC2 complex by Atomic Force microscopy (AFM).

51) ROLE OF THE FENESTRATIONS FOR THE BINDING OF A1899 TO TASK-1 POTASSIUM CHANNEL

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A1899 is a potent and highly selective antagonist of the two-pore domain potassium (K_{2P}) channel TASK-1. It was previously reported that A1899 acts as an open-channel blocker and binds to residues of the P1, P2 regions, the M2, M4 segments and the halothane response element. The recently described crystal structures of K_{2P} channels together with the newly identified side-fenestrations indicate that the drug binding site of TASK channels, initially proposed to be located purely in the central cavity, might need a re-evaluation. On the other hand, the side-fenestrations might represent a route for A1899 to access its binding site. In this study, we describe the binding mechanism of A1899 to TASK-1, using several computational techniques such as homology modeling, molecular dynamics simulations, molecular docking and binding free energy calculations, using recently available K_{2P} crystal structures as templates. We demonstrate that A1899 tightly binds to K_{2P} channels with open fenestrations and that A1899 cannot travel from the membrane phase through the fenestrations to reach this particular binding site.

52) TESTING THE PERFORMANCE OF DIFFERENT GPU BASED WORKSTATIONS RUNNING NAMD MOLECULAR DYNAMIC SIMULATIONS

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Molecular Dynamics Simulations (MDS) have become a powerful tool to study atomistically the behavior of many important biological molecular systems. Nevertheless, many of these processes take place on time scales much longer than those that can be studied by conventional CPU based workstations.With the advent of new technologies based on GPU computing, many algorithms involved in MDS can be adapted to GPU acceleration. Thus, nowadays many research groups may choose GPU-accelerated desktop workstations instead of a typical CPU clusters. For instance, the software Desmond (as a component of the Schrodinger suite) increases the performance up to 10 times faster in GPU relative to contemporary multi-core CPUs, rising simulation times significantly. Recently, the free software NAMD was implemented in GPU, but the performance has not been tested in most common arquitectures. This testing could contribute to get better simulation times by using the minimal computational resources. In this work, the performance of 6 different computer machines were evaluated, including 1 cluster and 5 conventional computers equipped with CUDA-capable GPUs (available in the Chilean market) carrying out MDS using the software NAMD 2.11 (released on December 2015). The simulated systems differ mainly in the number of atoms (system size) and the biological nature of them (globular and membrane proteins). The different architectures and different combinations of GPU / CPU were evaluated comparing their performances (ns / day) and their respective cost-benefits.

53) CLONING AND FUNCTIONAL CHARACTERIZATION OF MOUSE AGMATINASE

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Agmatine is a polyamine that functions as a neurotransmitter, is a precursor for polyamine synthesis, has hypoglycemic actions, and produces anticonvulsant, anti-neurotoxic and antidepressant-like effects in vivo. The key enzyme in regulating the physiological levels of agmatine is agmatinase that specifically hydrolyzes agmatine to putrescine and urea. Sequence alignment of agmatinase from different species indicates that the mouse agmatinase, differs in four of seven key residues that participate in its interaction with Mn2+ in the active site, which is essential for the hydrolysis of the guanidine group within members of the ureohydrolases. Therefore, murine agmatinase may not catalyze the hydrolysis of agmatine. To analyze its functional activity, we cloned agmatinase cDNA from mouse liver extracts and expressed it in Saccharomyces cerevisiae TRY104Δspe1. Agmatinase product, putrescine, is a precursor for polyamine biosynthesis, which is essential for cellular division, in vivo polyamine synthesis was examined by a complementation test in a S. cerevisie TRY104Δspe1 strain deficient in polyamine biosynthesis. The yeast cells transfected with mouse agmatinase were able to grow in the presence of agmatine, suggesting that murine agmatinase sustains polyamine synthesis in vivo for hydrolysis of agmatine. In addition, we generated four mouse agmatinase mutants that include amino acid residue that is necessary for Mn2+ stabilization into the active site. Each variant supported polyamine biosynthesis at levels similar to that observed for wild-type mouse agmatinase. It is possible that the interactions with Mn2+ ions increased in the mutants; however, an increase in yeast growth was not observed. VRID-Enlace 215.037.019-1.0

54) DETERMINATION OF STRUCTURAL CHARACTERISTICS OF 5´END OF MRNA OF INFECTIOUS PANCREATIC NECROSIS VIRUS (IPNV)

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Infectious pancreatic necrosis (IPN) is a contagious viral disease affecting salmonids, causing large economical losses in aquaculture worldwide. Its etiological agent is the IPN virus that belongs to *Birnaviridae* family. The unenveloped virions contain the viral genome that consists of two segments of uncapped and unpolyadenilated double-stranded RNA (dsRNA), dsRNA-A and dsRNA-B. Each 5' extreme of the viral RNA is covalently attached to the viral protein genome (Vpg). When Vpg is not linked to the genome acts as the viral RNA-polymerase RNA-dependent (Vp1). Vpg binds to RNA during RNA synthesis: free VP1 self-guanylates twice in a Ser residue. These guanidines align with two cytidines of the minus strand. Thus, Vpg acts as a primer for the synthesis of plus strand. This model suggests that both viral gRNA and mRNA are linked to Vpg. If Vpg is linked to the viral mRNA, it might have implications in its translation. However, there are only few studies about regulation of viral mRNA translation. In this work, to analyze the structural characteristics of mRNAs, we isolated the mRNAs directly from infected cells and from ribosomal fractions of infected cells. To determine if Vpg is linked to the viral mRNA, we performed immunoprecipitations with specific antibodies against Vpg and we detected the presence of +ssRNA, using specific primers. Additionally, we isolated viral mRNA-A and B, using specific probes attached to biotin. These results showed that viral mRNAs are linked to Vpg suggesting that this viral protein could be involved in the translation of IPNV mRNAs.

55) MITOCHONDRIAL DYNAMICS MODULATES CELL EXPANSION AND DIFFERENTIATION IN HUMAN HEMATOPOIETIC STEM CELLS.

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Introduction: Mitochondrial dynamics (MtDy), that regulates mitochondrial morphology, number and function through fusion and fission events is essential for cell physiology in many cell types and a failure in this process leads to cell death. Thus, we hypothesize that MtDy is occurring in erythropoiesis and required for the commitment, expansion and differentiation of hematopoietic stem-cells into red-blood cells.

Methodology: MtDy gene-expression was analyzed in mouse G1ER and Human cord-blood CD34+ cells during erythropoiesis by RT-PCR. Lentivirus-transduced CD34+ cells that over-express the mitochondrial fission protein FIS1, were induced to erythroid differentiation and followed by flow cytometry. Mitochondrial morphology and bioenergetics were also studied by confocal and TEM-microscopy.

Results: Gene expression of MtDy genes suggested a main role for the *fis1* gene throughout erythropoiesis. FIS1 over-expression (FIS1-OX) at early erythropoiesis arrested erythroid differentiation at the level of basophilic-erythroblast while increased the CD34+ and progenitors cell number. Bioenergetics analyses showed that FIS1-OX caused a reduction in $\Delta\Psi mt$ as well as in protein expression of respiratory complex II and IV. Confocal microscopy of FIS1-OX cells displayed rounder mitochondria than control ones. Furthermore, TEM-microscopy revealed an immature-like mitochondrial phenotype. Cyclosporine A treatment, an inhibitor of the mPTP, rescued erythroid differentiation in FIS-OX cells which involved the participation of the mPTP when mitochondrial fission is exacerbated. **Conclusions:** MtDy occurs in erythropoiesis and the right balance between fusion and fission events allows the appropriate erythroid differentiation. Exacerbated fission avoids mitochondrial maturation and promotes progenitor cell expansion while arrests cell differentiation through the involvement the mPTP.

56) TESTING THE HALOPHILIC CHARACTER OF THE ADP-DEPENDENT KINASES FROM THE *METHANOSARCINALES* GROUP OF ARCHAEA AND ITS EVOLUTIVE HISTORY

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Methanosarcinales comprises one of the two major groups of halophilic archaea whose organisms are able to grow at near saturating salt concentrations, incorporating high cations concentrations inside the cells. A described mechanism that allow proteins to deal with these salt concentrations is the intracellular accumulation of several organic solutes, being betaine the most common. However, to date there are no studies that evaluate the halophilic character of proteins from *Methanosarcinales* and the role of betaine in their properties. To address this issue we use the ADP-dependent phosphofructokinases (PFK-ADP) from *Methanosarcinales* as a model, where we can find the enzyme from *M. evestigatum* (extremely halophilic organism) and *M. mazei* (non-halophilic organism). In order to study the evolutionary nature of this trait we include the resurrected enzyme of the last common ancestor of the order *Methanosarcinales.* Kinetic experiments show that the two extant enzymes exhibit a similar dependence of their activity with both NaCl and KCl being inhibited over 0.5 M salt, while the ancestral protein remains active at higher salt concentrations. For the *Methanosarcinales* enzymes, inhibition was reverted in the presence of betaine enabling these enzymes to retain over 90 % of its maximum activity at 2 M salt concentration. Temperature dependent denaturation followed by circular dichroism show that extant proteins and ancestor are stabilized by molar concentrations of salt. These results suggest a halophilic nature of the ancestor and gives light about the mechanism by which *Methanosarcinales* proteins are fully active and folded at high salt concentrations.

57) POST-TRANSCRIPTIONAL CONTROL OF CRITICAL REGULATORS OF GENE EXPRESSION DURING HIPPOCAMPAL MATURATION

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The hippocampal maturation process requires expression of plasticity-related genes, as well as the silencing of inducers of non-neuronal cell lineages. Contributing to these processes are transcriptional and post-transcriptional epigenetic mechanisms, including DNA methylation, histone modifications, non-coding RNAs and microRNAs. We have described that during maturation of hippocampal neurons, the catalytic subunits of Polycomb Repressive Complex 2 (PRC2) exhibit a differential expression pattern. Ezh2 is found predominantly in immature neurons, whereas Ezh1 is expressed throughout the maturation process. To assess the mechanisms supporting these expression patterns, we analyze the global transcriptome and microRNA expression profile in mature and immature neurons. This as there is evidence indicating that critical regulatory components associated with hippocampal maturation can be down-regulated by microRNAs. We generated global miRNA-mRNA interaction network models to identify potential regulatory nodes. These strategies lead to the identification of differentially expressed miRNAs. Among them, miRNAs that can target the 3'-UTRs of mRNAs coding for Ezh1, Ezh2 and the plasticity-related gene PSD-95. Additionally, we assessed the epigenetic mechanisms that mediate silencing of non-neuronal specific genes. Our results indicate that in hippocampal cells Ezh1 and Ezh2 are critical components during PSD-95 gene expression regulation as well as during silencing of non-neural genes like the osteoblast master regulator Runx2. Moreover, we found that the expression Ezh1 and Ezh2 during hippocampal maturation is controlled by microRNAs mir-7a, mir-7b and let7e. Hence, our results establish a direct connection between post-transcriptional mechanisms mediated by microRNAs and the function of epigenetic mechanisms that operate in hippocampal tissue.

58) EVIDENCE OF THE PROTECTIVE EFFECT OF ANTIOXIDANT COMPOUNDS FROM PLANTS EXTRACTS IN A CELLULAR MODEL OF INFECTION BY *HELICOBACTER PYLORI.*

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The *Helicobacter pylori* (Hp) infection leads to gastroduodenal inflammation mediated by reactive oxygen species (ROS) as well as superoxide dismutase (SOD) and cyclooxygenase (COX-2) expression, both events being involved in the development of gastric cancer (GC). We evaluated the protective effect of phytochemicals and antioxidant from natural sources on the production of ROS and the expression of superoxide dismutase (SOD) in CG cells infected with (Hp). METHODS:Extracts of plants were tested for antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. The effects of extracts from plants, H_20_2 and Hp on gastric cancer lines AGS and SNU1,were evaluated according to cell viability. To evaluated the protective effects ofextracts from plants, the cells lines were pre-treated with extracts by 24 h. Then were exposed to H_2O_2 and Hp and the effect of each treatment on the cell viability, production of ROS and SOD expression was determined by MTS assay, 2',7'-dichlorodihydrofluorescein diacetate probe test and Western blot analysis, respectively. RESULTS. After 24 h incubation of SNU1 and AGS cells with extracts plants and infected with Hp, the viability was increased and the generation of ROS diminished. Under the same conditions, the expression of SOD was increased. CONCLUSIONS. The results observed in this study may be result from the antioxidative propertie in the extracts as flavonoids, tannins, carotenoids, etc., against generation of ROS induced by Hp. Accordingly, these molecules could have an anti-inflammatory role leading to attenuated cell damage by bacterial infection; this mechanism anti-inflammatory require to be elucidated.

59) MOLECULAR CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF DIFFERENT ECOTYPES OF *CURCUMA LONGA* OBTAINED IN EASTER ISLAND.

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Introduction. Curcuma longa (CL) or turmeric is a widely cultivated plant in South East Asia, with high medicinal and cultural value in India. The rhizome of the plant has medicinal properties, where the curcumin is responsible of biological activity. In Easter Island is widely used to treat various types of diseases, including cancer patients. The genetic diversity of CL in Easter Island is unknown and also its biological activity. The aim of this study is to characterize the genotype of CL present in Easter Island and evaluate the biological activity from extracts of CL in a gastric model cell.

Methods: Microsatellite or SSR (Simple Sequence Repeat) were utilized to characterize the genetic diversity of CL. The extracts were obtained from leaves and rhizome. The antioxidant activity was determine using DPPH reagent. In order to assess the viability and cytotoxicity of extracts, a MTS assay was performed on the cell line AGS.

Results: A total of 8 SSR were analyzed from five plants of CL. The extracts presented high content of polyphenols and high antioxidant activity. Also, the extracts shown antiproliferative properties on AGS cell lines.

Conclusion. The preliminary results indicated that CL from Easter Island have a potential biological activity that require to be elucidated. In a future the SSR amplified, will be analyzed by sequentiation to determine its phylogenetic relationship

60) Structural domains within the 5'leader of the HIV-1 full length mRNA and the ribosomal protein S25 influence cap-independent translation initiation

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The 5'leader of the HIV-1 genomic RNA is a multifunctional region that folds into secondary/tertiary structures that regulate multiple processes during viral replication, including translation initiation, thereby viral protein synthesis. In this work we examine the internal ribosome entry site (IRES) located in the 5'leader that drives translation initiation of the viral Gag protein under conditions that hinder cap-dependent translation initiation. Through a knock-down approach, we show that full activity of the HIV-1 IRES relies on the presence of the ribosomal protein S25 (eS25). Additionally, a mutational analysis revealed that the HIV-1 IRES is modular in nature, with a core region composed of the PBS, SD and DIS domains. Through mechanistic studies using the scanning-inhibitor drug edeine, we show that once the 40S ribosomal subunit is recruited to the IRES, translation initiates without the need of ribosome scanning. These findings elucidate a mechanism of initiation by the HIV-1 IRES whereby a number of highly structured sites present within the HIV-1 5'leader leads to the recruitment of the 40S subunit directly at the site of initiation of protein synthesis.
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61) THE TRANSCRIPTION FACTOR ZEB1 PROMOTES TUMORAL MALIGNANCY BY INDUCING THE EPITELIAL-MESENCHYMAL TRANSITION PROGRAM AND DOWNREGULATING THE ANDROGEN SYNTHESIS PATHWAY

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Prostate cancer (PCa) constitutes the third cause of cancer related deaths among men in Chile. It has been reported that one of the factors that promotes metastasis and tumoral progress is the epitelial-mesenchymal transition program (EMT). This process is regulated by transcription factors like ZEB1 and is characterized by a loss of cellular adhesions and a gain of migratory and invasive properties, nevertheless it is still not clear what changes can EMT induce in the tumoral cell steroidogenesis. In this study the prostate cancer cell line DU145 was used to study the effect of ZEB1 silencing on EMT and cellular steroidogenesis during prostate cancer. ZEB1 silencing was achieved using lentiviral transduction and EMT was assessed using qRT-PCR and western blot to analyze expression levels of Vimentin and E-Cadherin (key markers of the EMT program). Changes in enzymes from the steroids pathway CYP17A1 and 5α-reductase were studied using qRT-PCR and Western blot, also, androgen receptor and its splicing variant ARV7 expression was studied using these same methods. Finally, testosterone levels in the culture medium were determined using the ELISA method. The results showed that ZEB1 silencing caused a downregulation of Vimentin and upregulation of E-Cadherin as well as a downregulation of the steroidogenesis enzymes, its receptor and a lower level of testosterone production. These results point to ZEB1 as an important promoter of tumoral progress in PCa.

62) KINETIC AND BIOCHEMICAL CHARACTERIZATION OF HUMAN ADP DEPENDENT GLUCOKINASE

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In some archaea the glycolytic pathway shows modifications in the glucokinase (GK) and phosphofructokinase enzymes which uses ADP instead ATP as phosphoryl donor group. Recently, it has been reported the presence of an ADP dependent GK in *H. sapiens* (hADPGK) associate to endoplasmic reticulum membrane (RE) whose active site is oriented to the cytosol and could be involved in T cell activation. Aminoacid sequences alignment between GKs from archaea and hADPGK, show that the latter one exhibits an extra 53 aminoacid segment in the N-terminal that has not a structural equivalent in archaeal enzymes. This segment is postulated to be responsible for RE membrane anchoring through an amphipathic a-helix (residues from 1-22). Also a proline rich region present from residue 40-53 would provoke conformational restrictions affecting enzyme activity. In order to evaluate the implication of this extra segment in the catalytic properties of the enzyme two truncated proteins, Δ53hADPGK and Δ22hADPGK, were generated. In the first mutant the proline rich region and the amphipathic α-helix were removed, whereas in the second only the amphipathic α-helix. Kinetic studies show that the V_{max} of the Δ53hADPGK protein decrease 10 fold while the K_M value is similar to one reported for the wild-type enzyme. The V_{max} of the Δ22hADPGK enzyme diminished even more while the K_M value is maintained. Both mutant enzymes use preferentially ADP as phosphoryl group donor and Mg^{2+} as divalent metal cation. These results suggest that the extra 53 residues segment would be important for enzyme activity regulation.

63) INTERACTION OF C-TYPE LECTIN RECEPTOR DECTIN-2 WITH MOLLUSK HEMOCYANINS

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Mollusk hemocyanins from *Megathura crenulata* (KLH), *Concholepas concholepas* (CCH) and *Fissurella latimarginata* (FLH) induce a strong Th1 immune response when are inoculated in mammals, and therefore they have several uses as immunostimulants. However, immunological mechanisms leading to its remarkable immunostimulatory properties are poorly known. Glicosylations, a structural feature of hemocyanins, would play a key role in these effects. In this context, C-type lectin receptors (CLRs) present on antigen presenting cells (APCs) such as macrophages and dendritic cells (DCs) could participate in its recognition as a “highly mannosylated infectious agent”. Therefore, we propose that the dectin-2 receptor, which recognizes structures with high mannose content, one of the most abundant sugars in hemocyanins, is able to interact with them and participates in the activation of APCs leading to a proinflammatory milieu. Binding assays by indirect ELISA, showed dectin-2 was capable to interact with every hemocyanin, as well as furfurman, as positive control. The hemocyanins binding was inhibited in presence of D-(+)-mannose in a dose-dependent manner but not D-(+)-galactose, suggesting a specific recognition between dectin-2 and hemocyanins. Binding assays with chemically deglycosylated hemocyanins showed partial reduction in the binding to dectin-2. Finally, studies are in progress using cell lines and primary cultures of murine macrophages and DCs, to analyze the expression of dectin-2 and correlation with activation by hemocyanins. We concluded that dectin-2 is one of potential CLRs candidates to interact with hemocyanins on APCs, promoting its activation.

64) COMPUTER-AIDED IDENTIFICATION OF NOVEL NON-STEROIDAL MINERALOCORTICOID RECEPTOR MODULATORS

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Mineralocorticoid Receptor (MR) antagonists are widely used for treating a variety of cardiovascular disorders. However, concerns of side effects due to cross reactivity with other steroid hormone receptors such as the Glucocorticoid (GR), Androgen (AR) and Progesterone (PR) receptors highlights the need of novel non-steroidal specific MR modulators. With this aim, a combined ligand and structure-based virtual screening campaing was performed**.** The crystal structures of the ligand binding domains of AR, GR and PR in complex with their endogenous ligands and those from MR in complex with small molecules were retrieved from the PDB Databank. For each complex, shape-based queries and structure-based pharmacophore models were generated. An in-house database of ~3,000,000 commercially available compounds previously filtered by ADME/Tox constraints, was virtually screened against an ensemble of MR complexes and ranked according to the predicted binding score. 3000 hits from docking in the MR-complexes were further filtered using the other nuclear receptors derived shape-based query to refine the search, and the best 300 hits binding mode were analyzed. The combined shared feature pharmacophore model obtained with AR, GR, and PR structures obtained an AUC of 0.86 indicating that the shape-query is predictive and well able to separate the actives from the decoys. A final selection of 30 diverse compounds was selected to further biological assays in adipose and endothelial cell lines. Gene expression analysis of MR target genes by RT-qPCR identified several compounds that display significant effect over MR target genes expression and similar profile to that of Spironolactone.

65a) TARGETING POLYPHOSPHATE KINASE 1 (PPK1) IN *PSEUDOMONAS AERUGINOSA* PAO1: TOWARDS NOVEL ANTIVIRULENCE COMPOUNDS

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The interest of the pharmaceutical industry in developing novel antimicrobials is decreasing, as established screening systems that identify compounds that kill or inhibit growth of bacteria can no longer be used. In bacteria, inorganic polyphosphate metabolism is not essential for growth but polyP deficiency affects various cellular processes such as bacterial virulence and susceptibility toward antibiotics, including persistence. Consequently, Polyphosphate Kinase 1 (PPK1), the enzyme responsible for polyP synthesis in many bacterial pathogens, has been proposed as a potential drug target for novel antivirulence molecules. Herein, we report the discovery of antivirulence molecules targeting inorganic polyP synthesis in *P. aeruginosa* PAO1. PPK1Pa was produced in *E. coli* strain BL21-AI and purified by affinity chromatography. Enzyme kinetics was established using a DAPI-based fluorescence enzyme assay that allows the biological evaluation of drug-like compounds previously selected from virtual screening of the OpenNCI database. Hits were further assayed using *D. discoideum* as a host-pathogen model. Our screening approach allows the identification of 3 lead compounds that do not kill the bacterial pathogen, avoid affecting the host and its beneficial bacteria and disrupt the production of virulence factors that damage the host.

65b) ROLE OF INSULIN-LIKE SYSTEM IN THE REGULATION OF SOMATIC GROWTH OF THE CHILEAN MUSSEL (*MYTILUS CHILENSIS*) DURING NUTRITIONAL RESTRICTION

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Introduction: Chile is among the top 5 producer of mussels in the world, focusing a large percentage of production in *Mytilus chilensis* intensive aquaculture. In spite of its economic and social importance, research about the molecular mechanism of growth in this species is limited. One of the most important factors affecting the growth rate in bivalves is food availability. In vertebrates, growth is regulated principally by the insulin related-like system. However, its biological contribution in marine bivalve's growth remains uncharacterized. In this work, we evaluated the effects of nutritional restriction in the expression of insulin-like system components of M. chilensis

Methods: Adult mussels were obtained from the Centro de Investigación Marina de Quintay (CIMARQ) (V Region, Chile), subdivided and conditioned during 2 weeks before the trial in three tanks (control, fasting group, Picropodophyllin (PPP) group). Starving and PPP (IGF receptor inhibitor) groups were assayed for 4 weeks, recording weekly weight variations. Total RNA of *M. chilensis* mantle was obtained and RT-qPCR assayed for *insulin growth factor* (*igf*); *phosphoinositide 3-kinase (pi3k); protein kinase b (akt),* growth inhibitor *forkhead box protein (foxo)* and *e3 ubiquitin-ligases*.

Results and Conclusions: Nutritional restriction and PPP treatment induces a weight loss in mussels. In addition a significant down-regulation of *igf*, *pi3k*, *akt* was observed, however, no changes in *foxo* and *e3 ubiquitin-ligases* expressions were detected. Our result suggests that growth signaling pathway triggered by IGF system modulates anabolic and catabolic process in *M. chilensis* under nutritional conditions.

66) DIFFERENTIAL AGE-RELATED EXPRESSION OF TOLL-LIKE RECEPTORS IN *HELICOBACTER PYLORI*-INFECTED PATIENTS

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Introduction: *Helicobacter pylori* infection is the main cause of chronic gastritis and peptic ulcer disease worldwide, and is strongly associated with gastric adenocarcinoma. Toll-like receptors (TLRs) are essential for the body's recognition of *H. pylori* and the onset of an inflammatory response. TLR-2 and TLR-5 are crucial for *H. pylori*-induced NF-κB activation. Moreover, increased TLRs expression in the gastric mucosa has been correlated with *H. pylori* infection and gastric cancer progression. Nevertheless, *H. pylori* causes less inflammation and overall less mucosal damage in infected children in comparison to infected adults. In this study, we investigate whether TLR expression patterns in *H. pylori*-infected children differ from those of infected adults. **Methodology:** 36 symptomatic patients, referred for upper GI endoscopy by their physicians, were included in this study. *H. pylori* infection was determined by Rapid Urease Test. mRNA expression of TLR-2, 4, 5, 9 and IL-8 was determined in the gastric mucosa by qRT-PCR and normalized by GAPDH. **Results:** Children with *H. pylori* infection (n=9) showed decreased expression of TLR-2 and TLR-5 in comparison with infected adults (n=9) (p<0.05).Regardless of age, patients infected with *H. pylori* showed significantly increased IL-8 expression (p<0.05), and no induction of TLR-4 and TLR-9 expression, compared with uninfected children (n=9) and adults (n=9). **Conclusion:** Low TLR expression in childhood might contribute to the decreased mucosal damage observed in *H. pylori*-infected children compared to infected adults; however, this could also promote *H. pylori* persistence in the host.

67) Transcriptional and protein expression in subcutaneous fat from Chilota and Suffolk Down lambs grazing Calafatal

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This experiment was carried out to evaluate the breed effect on gene and protein expression of lipogenic key enzymes and one transcription factor in subcutaneous adipose tissue from back fat in lambs. Eight male Chilota and 6 male Suffolk Down lambs 2 mo age, uncastrated, no twins, were located to graze a “Calafatal”, a typical secondary succession at Butalcura Research Station, Chiloé Archipelago (Chile). After 62 d, lambs were slaughtered according to Chile’s meat industry standars. Fatty acid profile, qRT-PCR and Western blotting analysis from subcutaneous adipose tissue were performed.mRNA expression levels of lipogenic enzymes (ACC, FAS, SCD) and transcription factor (SREBP-1) gene expression did not significantly differ between both lamb breeds ($P > 0.05$), Chilota lambs showed higher sum PUFA (1291 ± 70.29 versus 1035 ± 88.91 mg/100g) and *n-6* PUFA (714.76 ± 35.37 versus 531.93 ± 44.73mg/100g) proportions and SCD index (55.69 ± 2.52 versus 45.39 ± 3.19 mg/100g) than Suffolk Down grazing the same pasture because of the lower ACC (0.35 ± 0.18 versus 2.33 ± 0.69 AU), FAS (0.16 ± 0.08 versus 1.10 ± 0.28 AU) and SCD (0.52 ± 0.09 versus 0.23 ± 0.13 AU) protein expression levels found in this breed. In summary, although gene expression was similar between breeds, the ACC, FAS and SCD protein expression levels can explain the lower *de novo* single SFA synthesis and higher sum PUFA and *n-6* PUFA proportions found in Chilota than Suffolk Down lambs.

68) ACTION OF γCDCPLI, A PHOSPHOLIPASE A_2 INHIBITOR FROM *CROTALUS DURISSUS COLLILINEATUS* SNAKE SERUM ON *LEISHMANIA (LEISHMANIA) AMAZONENSIS* PROMASTIGOTES

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Leishmaniasis are treated with drugs associated with high toxicity, parasite resistance and limited efficacy. Therefore, the development of new strategies for leishmaniasis treatment is essential and snake venoms are natural compounds with potential to yield novel drugs. In this study we evaluated the effects of γCdcPLI, a phospholipase A2 inhibitor from Crotalus durissus collilineatus snake serum, on viability and parasite-macrophage interaction of Leishmania (Leishmania) amazonensis. Viability assay was performed on promastigotes and macrophages cultivated in absence (control) or presence of γCdcPLI (0.78–100 µg/mL) up to 72 h by MTT assay. For invasion assay into macrophages, promastigotes previously incubated for 1 h in presence (10 and 50µg/mL) or absence of γCdcPLI (control) were added onto monolayer macrophages and incubated at 37°C in a CO2 incubator for 4h. After incubation, the cells were stained by Giemsa. The invasion assay was also performed with macrophages previously incubated for 1 h in the presence or absence of γCdcPLI. γCdcPLI it was cytotoxic for promastigotes showing IC50 of about 50µg/mL. Interestingly, the phospholipase A2 inhibitor reduced macrophages viability by only 30% at 50 µg/mL concentration. γCdcPLI interfered with the invasion capacity of promastigotes in macrophages, causing significant reductions of approximately 10–12% at all toxin concentrations tested. In addition, when the macrophages were previously incubated with γCdcPLI the invasion parasite capacity showed significant reductions of approximately 20–30%. Thus γCdcPLI is an important tool for the discovery of new targets on parasite and an alternative compound to improve the effectiveness of leishmaniasis treatment.

69) PROTEOMIC ANALYSIS OF *BOTHROPS PAULOENSIS* VENOM: STRUCTURAL INSIGHTS OF A PII SVMP

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The present work establishes a venom composition analysis of *Bothrops pauloensis* venomby LC/MSMS. We identified 153 sequence in the venom belonging to several toxin families including metalloproteinases (PI, PII and PIII subclasses), phospholipases A_2 (PLA_2s), serine proteinases, L-amino acid oxidases (LAAOs), bradykinin-potentiating peptides (BPPs), disintegrins and C-type lectins. The metalloproteinases (SVMPs) were the main group of toxins identified, corresponding to approximately 48% of the total spectra. The P-I SVMPs were predominant followed by P-III and P-II respectively. A peptidome analysis was performed for the first time with *B. pauloensis* venom and identified disintegrins, bradkinin potentiating peptides and fragments of larger proteins such as PLA_2, SVMPs and LAAOs. In addition, we generated, for the first time, *in silico* structural models for a P-II SVMPs identified in the venom. The predicted proteins displayed conservation of interaction sites of calcium and zinc ions, as showed by molecular dynamic simulations. The obtained results showed the abundance of biologically active components present in *B. pauloenis* venom, which may help to optimize the search for specific types of proteins.

70) DIFFERENTIAL RESPONSE OF TWO PUTATIVE WNT/β-CATENIN TARGET GENES, CX43 AND DAX1 IN 42GPA9 (MOUSE ADULT SERTOLI) CELL LINE.

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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 and Dax1 are important proteins for testicular development. These genes have TBEs (TCF binding elements) within their promoters and in transgenic mouse models, cx43 and dax1 are deregulated possibly affecting Sertoli cell functionality.

We evaluated whether this signalling pathway induces upregulation of CX43 and DAX1 geneexpression in 42GPA9 cells and the possible molecular mechanism involved in the differential response of these genes.

Nuclear translocation of β-catenin was evaluated by immunodetection. mRNA abundance was determined by RT-qPCR and histone marks and β-catenin promoter occupancy at the TBEs found in CX43 and DAX1 gene was assessed by ChIP analysis. Luciferase assays in an heterologous system (HEK293 cells) was used to study CX43 as a direct target.

Sertoli cells responded to treatments, accumulating β-catenin within the nucleus and activating *axin2* transcription. Stimulated 42GPA9 cells showed a 2-fold increase of *cx43* mRNA and a 2-fold increase of luciferase units in the heterologous system, while *dax1* mRNA was not affected. Histone marks of activation such as H3K9Ac and H3K4me3 were found only in CX43 TBE although β-catenin was recruited in both CX43 and DAX1 TBEs.

These findings suggest that CX43 gene is a direct target of β-catenin upon activation of this signaling pathway in 42GPA9 cells. FONDECYT 1141033

71) DOXYCYCLINE INDUCES THE MITOCHONDRIAL UPR AND INCREASES MITOCHONDRIAL METABOLISM 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 energy metabolism. 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 particularly susceptible to accumulation of misfolded/damaged proteins due to their pro-oxidant environment and the requirement of coordinated nuclear/mitochondrial gene expression. Mitochondrial UPR (UPR^{mt}) increases mitochondrial protein handling capacity, but it remains unknown whether this response involves metabolic changes. The aim of this work was to evaluate whether the response to mitochondrial proteotoxic stress involves an early metabolic component to support homeostasis. **Methodology:** We use doxycycline treatment (15-60 µg/mL) for 0-72 h to alter mitochondrial translation and induce proteotoxic stress in HeLa cells. Expression of mitochondrial and nuclear-encoded proteins was analyzed by Western blot. UPR^{mt} markers were analyzed by RT-qPCR. Oxygen consumption rate was measured with a Clark's electrode and 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 (CHOP, C/EBPβ, ClpP, mtHsp60) with a peak at 48 h of treatment. They also exhibited an increase of 20% in ATP levels and 50% in oxygen consumption rates between 2 and 4 h of treatment. **Conclusions:** Mitochondrial metabolism increases during the early stage of proteotoxic mitochondrial stress in HeLa cells. However, it remains unexplored the mechanism for increasing mitochondrial bioenergetics.

72) DESIGN OF NEW NANO-CARRIERS BASED ON BIOINFORMATICS ANALYSIS OF PROTEIN-DNA INTERACTIONS. MOLECULAR DYNAMICS AND EXPERIMENTAL VALIDATION.

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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 [1]. 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 [3]. 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 [2] . An efficient means for rational design of dendrimers as a carrier of a particular agent involves interaction among atomic-scale studies, chemical synthesis, and biological characterization. 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. 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. Finally, we developed new dendrimer-based nanoparticles and we carried out nucleic acid transfection experiments showing successful results in the increase of the efficiency over commercial reagents.

73) EVOLUTIONARY HISTORY AND COMPLETE REVERSAL OF COFACTOR SPECIFICITY OF MEMBERS OF THE 6-PHOSPHOGLUCONATE DEHYDROGENASE FAMILY

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In this work, structural and rational design studies of a dimeric $NADP^+$-specific (*Escherichia coli*) and a tetrameric NAD^+-preferent (*Gluconobacter oxydans*) 6PGDH were developed to understand the basis of substrate specificity and oligomeric evolution in this family. For both enzymes, ITC and kinetics experiments revealed a random sequential mechanism. Moreover, structural and phylogenetic insights show that dimeric 6PGDHs evolved from tetrameric enzymes through the duplication of the C-terminal domain, without altering the inter-subunitary contribution to the 6PG-binding site. The family is formed by two monophyletic groups, each containing conserved motifs at the $NAD(P)^+$-binding pocket in the N-terminal domain. While the presence of Asn followed by Arg in this motif seems to correlate with a $NADP^+$-preference, Asp followed by Val/Leu appears to correlate with NAD^+-preference. In the *Ec*6PGDH, the mutation N33D/R34V/K38A changes the 1715-fold preference for $NADP^+$ into 33-fold preference for NAD^+, and the variant N33D/R34V/S35K/K38N improved even more the catalytic efficiency for NAD^+, showing no detectable activity for NADP. For *Go*6PGDH, positions 30 and 31 modulate the cofactor preference: D30N changed the specificity from a 16-fold in favor of NAD^+ to an 100-fold $NADP^+$ preference. NAD^+ specificity quotient increased dramatically in the mutants R31V (40-fold) and R31L (1000-fold) as compared to *Go*6PGDH. This study allows an overall understanding of the evolution, substrate and cofactor specificity in the 6PGDH family that could be extended to other Rossmann fold dehydrogenases.

74) BOX B IS NOT CRITICAL FOR HIGH AFFINITY BINDING OF ROB PROTEIN WITH *MAR* AND *MICF* DUPLEX DNA

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MarA and Rob are two transcription factors from *E. coli* with common DNA binding domain (DBD) that consists of a bipartite Helix-turn-helix (HTH) motif. Rob has an additional regulatory domain at the C-terminus. Recognition helices, H3 and H6, from the DBD of MarA interact with the major groove in two regions of the target DNA (called marbox) defined as boxA (6 bp) and boxB (7bp), respectively. However, in the case of Rob, the interaction of H6 with boxB is absent, suggesting an alternative binding mode of DNA. To assess the role of H6 from bipartite HTH motifs in the specific recognition of marbox DNA, electrophoretic mobility shift assays were carried out. The union of MarA and Rob to different duplex DNA was analyzed, including *mar* and *micF* marboxes, as well as mutated and swapped sequences of these. We found that Rob and MarA specifically bind to both *mar* and *micF* marboxes, while no binding to DNA was observed when the boxes A and B were swapped. These results suggest that the orientation of boxes A and B in *mar* and in *micF* DNA is essential for specific binding by MarA and Rob proteins. However, these two proteins still retain specific binding to *mar* and micF duplex DNA when boxB is mutated, suggesting that only boxA would be necessary for high affinity binding and that two alternative different binding modes for Rob and MarA with marboxes may co-exist.

75) COTREATMENT WITH OLEIC AND LINOLEIC UNSATURATED FATTY ACIDS REGULATE MITOCHONDRIAL CONTENT IN SKELETAL MUSCLE: POSSIBLE ROLE OF AUTOPHAGY

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Overweight and obesity affect nearly 40% of the world's population. Skeletal muscle is one of the most important regulators of lipid metabolism, as its mitochondria consume large amounts of fatty acids (FA). Oleic and linoleic acids (OA and LA, respectively) are the most abundant unsaturated fatty acids in our diet and it has been shown they activate mitochondrial biogenesis. Nevertheless, little is known about the actual contribution of these FA on mitochondrial content in skeletal muscle and whether autophagy plays a role in this process. C2C12 myotubes were treated with OA or LA for 0 – 48 h and then mitochondrial respiratory complex levels, mitotracker green (MTG) fluorescence and mtDNA nucleoids were measured to evaluate mitochondrial mass. LC3-II levels and LC3-II/LC3-I ratio were determined as autophagy markers. As an *in vivo* approach, C57bl/6 mice were feed a high-fat diet (HFD) for 12 weeks and gastrocnemious and soleus muscles were extracted to perform the same analyses. Neither OA nor LA led to increased mitochondrial mass in myotubes or myoblasts. However, when used together, OA and LA increased mitochondrial proteins levels and MTG fluorescence after 6 h of stimulation. At the same time, LC3 processing was increased, suggesting activation of autophagy. Gastronemius, but not soleus muscle from HFD mice, showed increased mitochondrial mass. Interestingly, this type of muscle has a larger activation of autophagy than soleus. Together, these results show that treatment with OA – LA or HFD increases mitochondrial content in skeletal muscle, and this relates with autophagy induction.

76) PULLING ON SUPER PARAMAGNETIC BEADS WITH MICRO CANTILEVERS: SINGLE MOLECULE MECHANICAL ASSAY APPLICATION

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In this work we demonstrate that it is possible to trap and release a super paramagnetic micro bead by fixing three super paramagnetic micro beads in a triangular array at the sensitive end of a micro cantilever, and by simply switching on/off an external magnetic field. To provide evidence of this principle we trap a micro bead that is attached to the free end of single DNA molecule and that has been previously fixed at the other end to a glass surface, using the standard sample preparation protocol of magnetic tweezers assays. The switching process is reversible which preserves the integrity of the tethered molecule, and a local force applied over the tethered bead excludes the neighbouring beads from the magnetic trap. We have developed a quadrature phase interferometer which is able to perform under fluid environments to accurately measure small deflections, which permits the exploration of DNA elasticity. Our results agree with measurements from magnetic tweezer assays performed under similar conditions. Furthermore, compared to the magnetic tweezer methodology, the combination of the magnetic trap with a suitable measurement system for cantilever deflection, allows for the exploration of a wide range of forces using a local method that has an improved temporal resolution.

77) UNRAVELING THE CATALYTIC MECHANISM OF PHOSPHOFRUCTOKINASE-2 FROM *E. COLI*: A QM/MM THEORETICAL STUDY

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The ATP-dependent phosphorylation of fructose-6-phosphate, named phosphofructokinase (PFK) activity, is one of the most important steps in the glycolytic pathway; this is why it is highly regulated in a wide variety of organisms. *Escherichia coli* has two extensively studied isoenzymes with PFK activity; however, no previous theoretical approaches deeply have consideredthe reaction mechanisms by which these enzymes perform their activity until now. In the current contribution, we present a combination of the string method and path collective variables for the exploration of the free energy surface, associated with the phosphorylation mechanisms in the minor isoenzyme from *E. coli* named Pfk-2.

The barrier height differences, found at the end of our calculations, show that the dissociative mechanism is more favorable than the associative one. In the case of the former one, a metaphosphate intermediate is initially formed and then, a proton is transferred allowing a further nucleophilic attack to obtain the final products. Conversely, in the case of the latter one, the mechanism begins with a proton being transferred directly to the γ-phosphate and triggered from the aspartic acid residue in the active site. The structural analysis, at each point of both mechanisms, not only reveals the role of the aspartic acid but also of the lysine residue, which helps to stabilize the transition state of the most stable mechanism. Finally, analyses of the charge variations along both paths are discussed, revealing interesting information about the driving forces in each mechanism.

78) IDENTIFICATION OF NEW LNCRNAS DURING OSTEOGENESIS

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Long non-coding RNAs (lncRNAs) are a heterogeneous class of RNA pol II transcripts, longer than 200 nucleotides, 5`capped, polyadenylated, and poorly conserved among mammalian species. Several studies have shown the contribution of lncRNAs to different cellular processes, including regulation of chromatin structure, control of mRNA translation, regulation of gene transcription, regulation of embryonic pluripotency and differentiation. Although limited numbers of functional lncRNAs have been identified so far, the immense regulatory potential of these RNAs is already evident, emphasizing that a functional characterization of lncRNAs is needed. 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 for Illumina sequencing. 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, detecting cell-type specific distributions. A group of these lncRNAs showed reduced enrichment in other mesenchymal-derived tissues, including muscle. Importantly, the expression of these lncRNAs was confirmed in both osteoblastic and myogenic mouse cell lines, identifying specific expression profiles. Together our results provide evidence of a new set of lncRNAs with potentially relevant regulatory roles during osteoblastic differentiation.

79) GROWTH INHIBITION OF PLANT PATHOGENIC FUNGI BY *ASPERGILLUS FOETIDUS* MEDIATED SYNTHESIZED CDS NANOPARTICLE: A POSSIBLE MECHANISTIC INSIGHT.

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Nowadays study of the biosynthesis process of nanoparticles attracts the scientists due to its application in different fields like pharmaceutical, bioremediation etc. So it can be used as antimicrobial agent over the traditional drugs as documented in various research reports. In such cases, nanoparticle mainly interacts with proteins and DNA like macromolecules. In our present study, we have synthesized CdS nanoparticles using *Aspergillus foetidus*. The synthesized nanoparticles were characterized precisely by zeta potential measurements, dynamic light scattering, atomic force microscopy, high resolution transmission electron microscopy, energy dispersive X-ray and X-ray diffraction studies. Such CdS nanoparticle shows significant antifungal activities against different plant pathogenic *Aspergillus species*. To know the mode of action of antifungal activity, we further investigate the interaction studies between CdS nanoparticles against BSA, DNA (calf thymus DNA) as a model experiment. Our results support that *Aspergillus foetidus* mediated biosynthesized CdS nanoparticles of ~20-30 nm effectively interact with BSA and DNA. Therefore, the prepared fungal inhibitory protocol of CdS nanoparticle could be applied selectively for controlling the growth of plant pathogenic *Aspergillus species*.

80) DETERMINATION OF THE MOLECULAR ARCHITECTURE OF HETEROMERIC CONNEXIN30/26 HEMICHANNELS.

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Connexins (Cx) form hexameric hemichannels that transport small molecules, including ATP and glutamate, to mediate cell-to-cell communication and intracellular signaling. Loss or malfunction of these proteins has been associated with a variety of diseases, becoming of great relevance in the biomedical area. There are over 20 vertebrate connexins, and most cells seem to express more than one isoform. Biochemical and functional data demonstrate that hemichannels can be heteromeric – composed of more than one connexin isoform and that the gating and permeability properties are modulated by the heteromericity. However, the subunit stoichiometry and arrangement within heteromeric hemichannels is essentially unexplored. Heteromeric Cx26/30 channels were immunopurified from HeLa cells expressing a combination of haemagglutinin tagged Cx26 (Cx26-HA) and Cx30 or Cx30 (Cx30-HA) and Cx26. Presence of the heteromeric Cx26/30 hemichannels was confirmed by western blot analysis. Purified samples were imaged by air tapping mode atomic force microscopy (AFM). Molecular volumes of the Cx26-HA/30 and Cx30-HA/26 hemichannel complexes showed particle population centered at 337±10 nm^3 and 427±20 nm^3 respectively, which is within the volume range expected for their hexameric molecular weights. Ongoing studies aim to visualize Cx26/30 hemichannels decorated with anti-haemagglutinin antibodies via AFM to determine both the number of each subunit in the complex and its subunit arrangement.

81) PHOSPHATASE CALCINEURIN REGULATES INSULIN/AKT-DEPENDENT GLUCOSE UPTAKE IN SKELETAL MUSCLE CELLS

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(Sponsored by FONDECYT 1161156 (SL); FONDAP 15130011 (SL), CONICYT PhD Fellowship (MN))

Skeletal muscle plays a key role in glucose homeostasis. This tissue is responsible of up to 80% of glucose disposal induced by insulin in postprandial conditions, helping to maintain exquisite glycaemic control. Alterations in this process are related to development of insulin resistance and type 2 diabetes mellitus. The molecular mechanisms related to insulin resistance in skeletal muscle remains poorly understood. Calcineurin is a Ca^{2+}-dependent serine-threonine phosphatase related to different processes. A recent work shows that skeletal muscle specific deletion of calcineurin protects from high fat diet (HFD) - induced obesity and metabolic disturbance through a mechanism that involves preservation of mitochondrial function. In the present work, we evaluated the role of calcineurin on insulin signaling and glucose uptake (2-NBDG incorporation) in rat skeletal muscle derived cell line L6. Our results shows that short-term (60 min) incubation with calcineurin inhibitors FK506 and cyclosporine A increases insulin-dependent Akt Ser^{473} phosphorylation and glucose uptake in L6 myotubes. The overexpression of calcineurin inhibitor protein (CAIN) shows similar results. On the other hand, the overexpression of constitutively active form of calcineurin decreases insulin-induced Akt Ser^{473} phosphorylation. Interestingly, we also observed an increase in RCAN1.4 expression (a marker of calcineurin-NFAT pathway activation) in gastrocnemius muscle from HFD mice. In conclusion, these data suggest that calcineurin disrupts insulin response in skeletal muscle cells, unveiling a possible new mechanism related to insulin resistance.

82) ADENOSINE PROMOTES CHEMORESISTANCE BY REGULATING MRP1 AND MRP3 EXPRESSION IN NORMOXIA AND HYPOXIA CONDITIONS

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Introduction: glioblastoma multiforme (GBM) is the brain tumor with worst prognosis. GBM is highly chemoresistant due mainly overexpression and high activity of transporter proteins that extrude antitumor drugs from the cell, as multiple drug resistance associated protein-1 and -3 (MRP1 & MRP3). Glioblastoma stem-like cells (GSCs) or glioblastoma initiating cells are a subpopulation with extremely chemoresistant phenotype. These cells produce high levels of extracellular adenosine, a nucleoside associated to chemo-resistance by the expression of drug transporters. These levels are increased in hypoxic conditions, a fundamental characteristic of these tumors. The aim of this study is to demonstrate whether adenosine regulates the differential expression of MRP1 and MRP3 in normoxia and hypoxia, and evaluate its effects on chemoresistance. **Methodology**: GSCs were prepared from U87 human GBM cell line using conditioned media. Cells were incubated under normoxic (21% O2) and hypoxic (0.1% O2) conditions. mRNA and protein levels were measured by RT-qPCR and western blot, respectively. Activity of MRPs was measured by fluorimetric assay. Chemoresistance was measured by MTT viability assay under normoxia and hypoxia treatment in combination with vincristine, etoposide and/or doxorubicin. **Results:**In normoxia, adenosine promotes MRP1 expression/activity by activating adenosine receptor A_3 subtype (A_3AR). Under hypoxia conditions extracellular adenosine levels are higher compared with normoxic conditions and promotes MRP3 expression/activity by activating adenosine receptor A_{2B} subtype (A_{2B}AR). These expression promotes chemoresistance in treatment with vincristine, etoposide and doxorubicin. **Conclusions:** Adenosine promotes chemoresistance by regulating MRP1 and MRP3 expression in normoxia and hypoxia conditions, respectively.

83) TNF-α MEDIATED AUTOPHAGY REGULATES VASCULAR SMOOTH MUSCLE CELL PHENOTYPE SWITCHING

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Atherosclerosis is characterized by vascular smooth muscle cells (VSMC) dedifferentiation to a proliferative and migratory phenotype. This cardiovascular disease has a chronic inflammatory component with the presence of tumor necrosis factor-α (TNF-α) in the atherosclerotic tissue. Furthermore, it has been observed that VSMC of atheromatous plaques have increased autophagy, a mechanism associated with protein and intracellular organelles degradation. Whether TNF-α induces VSMC dedifferentiation, and if this phenotype switching is mediated by autophagy remains unexplored. Studies were performed in a rat aortic VSMC cell line A7r5. Cells were stimulated with TNF-α 100 ng/mL. Autophagy was determined by measuring LC3-II and p62 protein levels. Autophagy was genetically inhibited with a siRNA Beclin1. Cell dedifferentiation was evaluated by measuring the expression of contractile proteins α-SMA and SM22, extracellular matrix protein osteopontin and type I collagen levels. Cell proliferation was measured by [^{3}H]-thymidine and MTT assay, and migration was evaluated by wound assay and transwell using a Boyden chamber. TNF-α induced autophagy as determined by LC3-II level increase (1.91±0.21, $p<0.01$) and p62 level decrease (0.77±0.05, $p<0.001$), as compared to control. In addition, TNF-α induced migration (1.45 ± 0.09, $p<0.01$), proliferation (2.33±0.24, $p<0.05$), extracellular matrix proteins collagen type I (3.09±0.85, $p<0.01$) and osteopontin (2.32±0.46, $p<0.05$), and decreased contractile proteins α -SMA (0.74±0.12, $p<0.05$) and SM22 (0.54±0.01, $p<0.05$). When autophagy was genetically inhibited, these TNF-α-induced phenotypic changes did not occur. In this study it was shown that TNF-α induces cell dedifferentiation, proliferation and migration in VSMCs in an autophagy dependent manner.

84) REGULATION OF AUTOPHAGY BY GLUCAGON-LIKE PEPTIDE I IN VASCULAR SMOOTH MUSCLE CELLS

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Alterations vascular smooth muscle cell (VSMC) dedifferentiation play a critical role in the pathogenesis of cardiovascular diseases, such as hypertension and atherosclerosis. Autophagy in VSMCs has been associated with contractile protein degradation. Glucagon-like peptide 1 (GLP-1) is an incretin used in diabetes type 2 treatment that shows cardioprotective actions. We propose that GLP-1 regulates VSMC dedifferentiation by inhibition of autophagy. To test this hypothesis, aortic VSMC cell line A7r5 were incubated with medium + 2% serum 24 h before experiments. Glucose deprivation using RPMI medium induced A7r5 autophagic flux at 4 h, evaluated by LC3II/LC3I ratio and p62 levels by Western blot, in the presence and absence of chloroquine (30 μM). The same result was obtained by determining autophagosome formation using LC3-GFP and fluorescence microscopy. GLP-1 (100 nM) inhibited autophagic flux as determined by LC3II/LC3I ratio, p62 levels and autophagosome formation. Our results showed that GLP-1 inhibits glucose deprivation-induced autophagy in VSMCs. GLP-1-dependent autophagy inhibition could be a new therapeutic effect of incretins.

86) ALTERED ADENOSINE SIGNALING AFFECTS RENIN-ANGIOTENSIN SYSTEM PEPTIDES REPERTORY IN DIABETIC NEPHROPATHY.

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INTRODUCTION. Progression of diabetic nephropathy (DN) is linked to intrarenal induction of the renin-angiotensin system (RAS) as well as increased levels of adenosine. The activity of RAS is dependent on the activity of peptidases that process the precursor angiotensin 1, such as aminopeptidase A (APA), generating products with poorly characterized effects on tissues and organs. Our aim was determine the role of adenosine and its receptors on RAS activity.

METHODS. Rat glomeruli were treated ex vivo with 10μM adenosine and pharmacological modulators of adenosine receptors for 24 h. Changes in protein levels of APA were determined by western blot. Physiological effects after in vivo administration of the adenosine A2B receptor antagonist MRS1754 (2,5 mg/kg/1 month), angiotensin 2-7 (Ang 2- 7, 300μg/Kg/day for 15 days) and angiotensin 2-10 (Ang 2-10, 300μg/Kg/day for 15 days) were evaluated in healthy and diabetic rats.

RESULTS. In glomeruli from diabetic rats ex vivo adenosine induced APA through A2B receptor. An antagonist of adenosine A2B receptor (MRS1754) blocked APA induction in DN. In vivo administration of RAS peptides generated from APA activity on angiotensin 1, had deleterious effects on rat physiology. Ang 2-10 increased blood pressure and Ang 2-7 mediated renal injury inducing the fibrotic marker αSMA and decreased urinary creatinine.

CONCLUSION. Adenosine altered peptides repertory of RAS by inducing renal aminopeptidase A. Peptides derived from APA activity had deleterious effects on renal function.

87) COMPUTATIONAL STUDY OF THE ADSORPTION MECHANISM OF IGG1 ON A KAOLINITE SOLID SURFACE USING ALL-ATOM MOLECULAR DYNAMICS TECHNIQUES

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Adsorption is a physicochemical interface phenomenon where particles interact over a surface inducing structural and functional changes. In case of proteins, the structural change affects their spatial configuration and stability. Due to its relevance in many biological and biotechnological processes, it is important to know those physicochemical factors involved which are still unclear at atomic resolution.

By using all-atom molecular dynamics techniques, it was characterized the protein adsorption behavior at single-molecule level. In this work, the model was developed using the IgG1 human antibody and a kaolinite solid surface. The simulation was made using NAMD and INTERFACE Force Field.

The protein model was built making a homology modeling, using as template the IgG1 sequence and its structure 1HZH.pdb from the Protein Database, conserving its protonated N-terminus and unprotonated C-terminus as well as all histidine residues in the unionized state. On the other hand, the solid surface of kaolinite was created by a script designed in the Perl programming language. This surface consists in a single layer extended of 38x22x1 units cell, equivalent to 200x200x200 A^3.

After 10 ns of simulation, a kinetic analysis of the adsorption process was made regarding protein properties such as charge, size and structural stability, surface properties including polarity and hydrophobicity, and environmental properties such as temperature, pH, ionic strength and buffer composition. These simulations represent novel insights into the adsorption mechanism that likely will guide interpretation of experimental results commonly observed at the single protein adsorption level. Funded by PCI-REDES150151.

88) THE TRANSCRIPTION FACTOR ZEB1 PROMOTES TUMOR PROGRESSION AND CHEMORESISTANCE IN PROSTATE CANCER CELL LINES

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It has been reported that one of the factors that promotes tumoral progress is the anormal activation of the epitelial-mesenchymal transition program (EMT) which allows tumoral cells to acquire invasive and malignant properties. The transcription factor ZEB1 is one of its main activators and has a higher expression in prostate cancer (PCa) samples with high Gleason score. Here we show that overexpression of ZEB1 promotes EMT, malignancy and chemoresistance in PCa cell lines. To achieve this we overexpressed ZEB1 in the 22RV1 PCa cell line and silenced it in the DU145 line using lentiviral vectors. Induction of EMT was evaluated by measuring the levels of the key markers E-Cadherin and Vimentin using qRT-PCR and Western Blot. Malignancy was evaluated using the boyden chamber assay, wound healing and a clonogenicity test. Sensitivity to Docetaxel was analyzed using the MTT method. The results showed that overexpression of ZEB1 was able to induce EMT by upregulating the mesenchymal marker Vimentin and downregulating the epitelial marker E-Cadherin. Contrary to this, ZEB1 silencing repressed Vimentin expression and upregulated E-Cadherin. The malignancy tests showed that overexpression of ZEB1 conferred enhanced motility and invasiveness and a higher colony formation capacity to 22RV1 cells whereas DU145 with ZEB1 silenced showed a decrease in those same properties. Finally, cells overexpressing ZEB1 exhibited a lower sensitivity to Docetaxel while silenced cells proved to be more sensitive. The results show that ZEB1 could be a key promoter of tumoral progress and resistance to treatment in advance stages of PCa.

89) DIFFERENTIAL EXPRESSION OF EPITHELIAL TO MESENCHYMAL TRANSITION TRANSCRIPTION FACTORS IN BREAST CANCER

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Breast cancer is the leading cause of cancer death among women worldwide, being distant metastases the main cause of death. Epithelial to mesenchymal transition (EMT) has been implicated in promoting cancer invasion and metastasis. TGF-β is a promoter of EMT by inducing the expression of transcription factors TWIST, SNAIL, SLUG and ZEB1. The expression of these transcription factors has been analyzed in several cell lines, although no studies have been performed in breast cancer tumors with different prognosis. In this study, we analyzed the expression of TWIST, SNAIL, SLUG and ZEB1, after TGF-β treatment in HCC1937 (basal-like) and T47D (luminal A) breast cancer lines, by immunocytochemistry. Additionally, these transcription factors were evaluated in 60 breast cancers tumors by immunohistochemistry. Our results showed increased nuclear expression of TWIST, SLUG and ZEB1 in HCC1937 cells, while TGF-β induced expression of SNAIL and SLUG in T47D cells. For breast cancer tumors, 40% presented expression of EMT transcription factors of which 68% were positive for lymph node metastases. TWIST, SNAIL, SLUG and ZEB1 were expressed in 22%, 30%, 30% and 70% of tumors, respectively. The majority of luminal A tumors showed expression of SNAIL (71%) while most of the basal-like tumors showed expression of TWIST (75%) and SLUG (71%). In conclusion we found differential expression of EMT transcription factors between basal-like and luminal A tumors, in concordance with our observations in T47D and HCC1937 cell lines, respectively. Our results suggest a different mechanism of EMT induction by TGF- β in tumors with different histopathology.

90) URINARY EXOSOMES EVIDENCE ALTERATIONS ON ADENOSINE METABOLISM DURING DIABETIC NEPHROPATHY.

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INTRODUCTION. Diabetic nephropathy (DN) is recognized by epithelial tubule cells injury and fibrosis. Further, progression of DN is associated with increased adenosine levels. Our aim was characterize urinary exosomes from DN patients and its correlation with altered adenosine metabolism and phenotypic changes on epithelial cells. **METHODS.** Urines samples were collected from control (HbA1c 4-6%), diabetic (HbA1c > 7%) and diabetic nephropathy patients (HbA1c > 7% y ACR > 300 mg/g) controlled at the CENAIA-Valdivia. The exosomes were purified by seriated ultracentrifugation to 120.000x*g*. The presence of CD73 and exosome markers was determined by western blot. HK2 cells were exposed to urinary exosomes and the induction of fibronectin was evaluated by western blot and cell viability was quantified by MTT reduction assay. **RESULTS.** The CD73 content was considerably higher in urinary exosomes from DN patients than those found in control and diabetic patients without nephropathy. The induction of the mesenchymal transition marker fibronectin, was observed in tubular epithelial cells exposed to exosomes from both diabetic and DN patients. However, cell viability only was decreased by 50% when exposed to exosomes from DN patients. **CONCLUSIONS.** Urinary exosomes from DN patients evidence intrarenal CD73 induction, related to increased adenosine levels during DN progression. Also, urinary exosomes transport factors associated with profibrotic activation and depletion of tubule epithelial cells.

91) NEW DNA STAINING IN GELS WITH TETRAZOLIUM SALTS IMPROVES INTEGRITY OF RECOVERED DNA

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The separation and visualization of DNA by electrophoresis is associated with a variety of analytical and diagnostic assays. Visualization is carried out through different methods; among them highlights silver staining and fluorescent dyes like ethidium bromide (EB) and SYBR Green I (SGI). Using fluorescent dyes allows recovery of the analyte and is more selective than silver, but requires instruments such as a transilluminator or fluorimeter to visualize the DNA. Here we described a new and simple method that allows DNA visualization to the naked eye by generating a purple formazan precipitate. It works by soaking the acrylamide or agarose DNA gel in a SG I and nitro blue tetrazolium (NBT) solution that, when exposed to light, produces formazan, as identified by mass spectrometry. A calibration curve made with a DNA standard established a detection limit of approximately 60 pg/band at 500 bp. The selectivity of the assay using different biomolecules showed a high selectivity for DNA. Integrity and functionality of the DNA recovered from gels was determined by enzymatic cutting with a restriction enzyme and by transforming competent cells after the different staining methods, respectively. Our method showed the best performance among the dyes employed. Based on its specificity, low cost and its adequacy for field work, this new methodology has enormous potential benefits to research and industry.

92) EFFECT OF NITROGEN DEFICIENCY IN THE PHENYLALANINE AMMONIA-LYASE (PAL) ACTIVITY AND IN THE CONTENT OF PHENOLIC COMPOUNDS IN *COLOBANTHUS QUITENSIS* (KUNTH) BARTL.

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Colobanthus quitensis is the only dicot of Antarctic territory, in field, some authors describe phenolic compounds as important molecules to control oxidative stress derived of environmental conditions and as UV-absorbing molecules. The phenolic compounds are synthesized from phenylalanine, so its synthesis depends of nitrogen status. Our aim is study the effect of the nitrogen deficiency in the phenylpropanoid pathway of *C. quitensis*. After fifteen days in nitrogen deficient growth conditions, we observed no apparent signs of stress, and we found a decrease in total phenolic content in aerial and radicular tissue, due with an inhibition in phenylalanine ammonia-lyase (PAL) activity, this inhibition is accompanied with a decrease in chalcone synthase (*CHS*) transcript levels and a significant decrease in total protein content in leaves. In the other hand, the primary enzymes of nitrogen metabolism, that mediate mobilization of nitrogen from old to young leaves, such as glutamic oxaloacetic transaminase (GOT), glutamic pyruvate transaminase (GPT), ferredoxin dependent-glutamine oxoglutarate aminotransferase (Fd-GOGAT), NADH dependent-glutamine oxoglutarate aminotransferase (NADH-GOGAT), NADH dependent-glutamate dehydrogenase (NADH-GDH) and NADPH dependent-glutamate dehydrogenase (NADPH-GDH) are highly induced by nitrogen deficiency treatment. Our results suggest a tolerance to nitrogen deficiency in *C. quitensis*, possibly due to an a remobilization of nitrogen from old to young leaves and owing to an privilege of using nitrogen in the primary metabolism above the secondary.

93) VIMENTIN AND E-CADHERIN EXPRESSION IN RELATION TO LYMPH NODE METASTASIS, IN BREAST CANCER TUMORS EXPRESSING MICRORNAS TARGETING TRANSCRIPTION FACTORS INVOLVED IN EPITHELIAL-MESENCHYMAL TRANSITION

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Breast cancer is the leading cause of cancer-associated deaths in women worldwide. 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. MicroRNAs are small non-coding RNAs whose expression has been demonstrated to be altered in different cancer types, including breast cancer. Because their ability to regulate large sets of genes involved in cancer growth and metastasis, microRNAs have emerged as candidate molecular biomarkers and novel therapeutic targets. In this work we used microarray microRNA expression data to identify microRNAs differentially expressed in breast tumors with lymph node metastasis. We used total RNA from 31 fresh frozen tumors, 16 obtained from patients with lymph node metastasis. Microarray analyses revealed 12 microRNAs differentially expressed between tumors with different lymph node status ($p<0.05$). In silico analysespredicted that these microRNAs regulate specific transcription factors that promote metastasis (SNAIL, SLUG, ZEB1 and TWIST1), regulating epithelial-mesenchymal transition proteins such as E-cadherin and Vimentin. The predicted targets are being tested by Luciferase reporter assays. As these transcription factors repress the expression of E-cadherin and promote vimentin expression, we evaluated the expression of these proteins in 22 breast tumors by immunofluorescence. We found that 6/12 tumors with lymph node metastasis have lost E-cadherin expression, and vimentin was expressed only in tumors that present lymph node metastasis. Our results suggest that differentially expressed microRNAs are regulating E-cadherin and vimentin in breast tumors

94) ABSCISIC ACID MEDIATES THE DESICCATION TOLERANCE IN THE ANTARCTIC MOSS *SANIONIA UNCINATA*

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The desiccation tolerance is defined as the ability of plants to recover their metabolism after having been subjected to a drying event (5-10% water content). It has been postulated that tolerance mechanisms are primitive because it is a common feature in the first lineages of plants, like mosses whose origin was 504 million years ago during the Ordovician period, an inhospitable period due to numerous events of drought and low humidity. Currently one of the most extreme environments, is the maritime Antarctic territory, due to high levels of ultraviolet radiation, salinity, poor nutritional soil conditions, low temperatures and strong winds, these environmental conditions restrict the development of vascular plants, but not the development of non-vascular plants like bryophytes, that are more abundant and diverse one of the most abundant moss is *Sanionia uncinata* (Amblystegiaceae), however, the mechanisms that allow tolerance to this environment have not been described. Our results show that *S. uncinata* accumulates the phytohormone ABA allowing to the moss tolerate the Antarctic environment. In laboratory conditions, ABA reduced the dehydration rate and also the oxidative damage on the other hand the treatment with NGDA (ABA biosynthesis inhibitor) increase the ROS levels and also membrane lipoperoxidation. Furthermore, ABA induces expression of low molecular weight dehydrins proteins and compatible osmolytes such as proline and glycine betaine, which are capable of protect the moss during desiccation events, explaining in part the biochemical mechanisms of *S. uncinata* to tolerate desiccation.

95) FAM162A: A NEW MITOCHONDRIAL PROTEIN RELATED TO MITOPHAGY IN MAMMALS

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Mitochondrial turnover is essential for cell physiology. In this process, selective mitochondrial autophagy called mitophagy plays a major role by removing old/damaged mitochondria from the mitochondrial network. Through the study of erythropoiesis, our laboratory found out that FAM162A, a mitochondrial protein involved in hypoxia-induced apoptosis, displays a gene expression pattern similar to LC3 and NIX, two known proteins involved in mitophagy. Interestingly, NIX was initially described as a pro-apoptotic protein. Thus, we hypothesize that FAM162A plays a role in mitophagy (as a mitochondrial receptor of the autophagic machinery). HEK293 cells were co-transfected with siRNA-FAM162A and GFP:mCherry-LC3 constructs to measure the autophagic flux. Mitochondria co-localization with autolysosomes was determined by co-staining with Mitotracker and Lysotracker dyes. Mitochondrial morphology and membrane potential were visualized by TMRE staining and epifluorescent microscopy. Cell viability was determined by Trypan blue. FAM162A knockdown causes a significant accumulation of autolysosomes suggesting a late-impairment of autophagy. As expected, an increased number of mitochondria co-localizes with autolysosomes. Furthermore, mitochondria become depolarized and fragmented. Cells die 48 hrs post-transfection with the siRNA-FAM162 construct. Knock-down of either mitochondrial receptor for the autophagic machinery such as NIX or proteins involved in mitochondrial fission which are needed for autophagy; or a deficiency in autophagy itself, have all in common to produce an accumulation of both depolarized mitochondria and autolysosomes. Thus our results strongly suggest that FAM162A has a role in mitophagy.

96) EFFECT OF THE FORCES INVOLVED IN THE CONFORMATIONAL CHANGES ASSOCIATED TO THE LIGAND BINDING AND CATALYSIS IN ADENYLATE KINASE.

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Enzymatic function and catalysis depend upon a fine balance between structural rigidity and flexibility. Although catalysis is not only determined by chemical reaction steps but also by protein conformational changes, it still remains unknown how enzymes achieve high catalytic efficiency. A modern approach to address this question is to consider enzyme catalysis and protein dynamics as mechanical processes, where forces and energy can be determined to study them. We are using adenylate kinase (AK) as our model to quantitatively asses the importance of the strain-induced theory and to study the cracking phenomena (local unfolding/refolding event during catalysis). Considering the 2RH5 AK structure, site-directed mutagenesis cysteine residues were introduced on each of the two domains of the protein, establishing specific chemically active sites for the covalently coupling of DNA strands to perform *in singulo* and *in multiplo* force experiments. *In singulo* unfolding force experiments by optical and magnetic tweezers manipulation, coupled to fluorescence, show that AK unfolds around 20 pN and has a reversible unfolding of 4 nm that could correspond to the Lid domain. Moreover, we constructed an AK-DNA chimera, attaching a single stranded DNA to AK which will function as an externally controllable molecular spring, by the application of an external force to control protein activity. Upon hybridization with a complementary strand, the DNA will substantially rigidify and the semi-rigid double stranded DNA will bend because of the constraint, exerting mechanical stress and altering AK conformation and activity. This novel *in multiplo* approach is called “the spring probe approach”.

97) METABOLIC ENGINEERING OF SWEET PROTEINS THAUMATIN AND BRAZZEIN IN KIWI (*ACTINIDIA DELICIOSA* VAR. HAYWARD) AND TOMATO (*SOLANUM LYCOPERSICUM* VAR. MICROTOM*)* FRUITS.

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Chile is the third largest exporter of kiwis in the world. 90% of kiwifruit exported by Chile correspond to the Hayward variety, which produces green fruit pulp that are widely known for their acidity. Therefore, we realized the opportunity to add value to these fruits, through increasing the sweetness without adding calories. To achieve this proposal, we used metabolic engineering of thaumatin (TAU) and brazzein (BRA) sweet proteins in kiwi fruits and tomato. These are non-toxic proteins which have 2000 to 3000 times more sweetness than sucrose and 10 times more sweetness than stevia. At present, they have been used as sweeteners and flavor enhancers in different meals and unlike stevia, they have a taste similar to sucrose. To accomplish this objective, we used a bicistronic expression system, allowing the expression of both proteins in a single reading frame separated only by the coding sequence of the viral self-cleaving 2A peptide, which allow the cleavage between genes upstream and below its sequence. In this work we also present evidence on vector functionality evaluation by means of transient expression in tobacco leaves and tomato fruits. Tomato and tobacco plants were stable transformed with the TAU-BRA vector and transgenic plants were selected to evaluate the expression and TAU-BRA abundance in fruits and leaves. Simultaneously, transformation of kiwi is also achieved and transgenic shoots with sweet proteins are under analysis.

98) GROWTH RESPONSE TO ALTERED NAD(P)H PRODUCTION BY THE PENTOSE PHOSPHATE PATHWAY IN ANAEROBIC CULTURES OF ESCHERICHIA COLI

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The capacity of metabolism to adapt to environmental and genetic perturbations is a fundamental biological phenomenon. In the facultative anaerobe Escherichia coli (E. coli) the absence of oxygen alters the maximum growth rate and changes the redox state of the cell in terms of the intracellular concentrations of NAD(P)(H). Also, genetic perturbations affecting metabolic flux distributions could affect the redox state. This occurs in E. coli with the loss of the pgi gene, encoding the phosphoglucoisomerase, that results in rerouting all the carbon flux through the oxidative branch of the pentose phosphate pathway (oxPPP) when glucose is the sole carbon source. In turn, this leads to an excess of NADPH that strongly decreases the maximum growth rate.In this work, we studied under anaerobic conditions the growth behavior of E. coli strains bearing the deletion of pgi (Δpgi) and the replacement of the native glucose-6-phosphate dehydrogenase (G6PDH) by an NADH-producing variant. Contrary to the expected decrease (ca. 40%) of wild type E. coli, the growth rate of Δpgi shows no major changes. We also observed a growth rate decrease upon cofactor switch of G6PDH, indicating that oxPPP might be operative in this strain under such conditions. Our results suggest that unbalance of NADPH could be managed by E.coli under anaerobic conditions, likely because of the presence of the transhydrogenase UdhA and an increased availability of fermentation pathways.

99) MODULATION OF MURINE DENDRITIC CELL FUNCTION BY A HERPES SIMPLEX VIRUS 2 DELETED FOR GLYCOPROTEIN D.

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Herpes simplex virus 2 (HSV-2) is the primary cause of genital ulcers worldwide and one of the most prevalent sexually transmitted infections in the world. HSV-2 negatively modulates the function of dendritic cells (DCs), interfering with their maturation process, stimulating the release of pro-inflammatory cytokines and ultimately eliciting their apoptosis. These effects directly impact on the ability of these cells to activate T cells. HSV-2 glycoprotein D (gD) is a surface protein that has been mainly described as a key viral determinant needed for viral entry, promoting the virus fusion at the surface of susceptible cells. Here, we show that HSV-2 genetically deleted for this glycoprotein, yet phenotypically complemented is attenuated in DCs, which are significantly more viable than DCs infected with wild-type HSV-2. DCs pulsed with the mutant virus promoted significant activation of virus-specific CD8+-T cells (GBT-I) and antigen-specific CD4+-T-cells (OT-II). In addition, the HSV-2 mutant promoted DC maturation and increased the secretion of IL-6 by these cells, whereas the opposite was observed with wild-type HSV-2. To assess the mechanisms by which the HSV-2 mutant virus promotes DC function, we assessed the unfolded protein response, the generation of reactive oxygen species and autophagy in these cells.

100) F26 AND F379 ARE IMPORTANT FOR SUGAR BINDING IN THE HGLUT1 HEXOSE TRANSPORTER

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The 3D structure of the hGLUT1 glucose transporter (Deng *et al., Nature* ***510***, 121, 2014) shows a substrate-binding cavity, which is built of polar residues establishing hydrogen bonds with the sugar, and hydrophobic residues assisting the correct orientation of the substrate within the pocket. In the model, residues F26 and F379 are perpendicularly positioned respect to the bound glucopyranoside ring, which suggests that they are either involved in substrate selection or allow the substrate to slide along the translocation channel. We tested this hypothesis by analyzing the functional consequences of replacing these residues by coupling site-directed mutagenesis with expression of the mutants into Xenopus oocytes. Our results indicate that changing the polarity of the lateral chain of both residues causes an acute loss of function and perturbs substrate affinity. These data support the notion that these phenylalanine residues are important determinants of the structure of the sugar binding site in the hGLUT1 transporter.

101) IDENTIFICATION OF CRUCIAL RESIDUES FOR EXTERNAL GATE CLOSURE ON THE HGLUT1 HEXOSE TRANSPORTER

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The glucose transporter GLUT1 maintains basal and constant glucose uptake in most tissues. This carrier, which belongs to the family of facilitative GLUT transporters, is indisputably the most studied transporter because of its essential role in providing glucose to the central nervous system to sustain the high energy requirements. Also, high expression levels of GLUT1 have been found in various types of cancers. The recent report of GLUT1's crystallographic structure (Deng et al., Nature 510, 121, 2014) has allowed us to relate the functional information with the structural dynamics of the protein. This structure suggests the existence of an exofacial gate that includes residues S294, N34, T310, and Q172, which occludes the ligand binding site from the extracellular environment. To test their participation in GLUT1's transport mechanism, we analyze the functional properties of single mutants of these residues after expression in Xenopus oocytes. Replacement of most of these residues resulted in a significant loss in transport activity. The effect is mainly explained by a lower catalytic efficiency of the mutants, without significant alteration of the KM values and sensitivity to GLUT1 classic inhibitors. The results demonstrate that these residues are critical for the activity of the transporter, supporting the concept that these residues interact with each other to form a putative exofacial gate in in hGLUT1 transporter.

102) IDENTIFICATION OF RESIDUES CONFORMING THE EXTRACELLULAR GATE OF THE HGLUT2 HEXOSE TRANSPORTER

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GLUT2 is a low affinity glucose transporter, manly expressed in liver, kidney, intestine, and pancreatic beta cells, fulfilling absorption and sensing functions. Recently, Deng et al. (*Nature* ***510***, 121, 2014) determined the crystal structure of GLUT1, a closely related glucose transporter, and identified some residues that could conform a gate that occludes the ligand binding site from the extracellular face of the transporter. These residues are conserved in GLUT2 and they are located in the same positions in a theoretical model of GLUT2. Here, we tested whether these residues play a structural and functional role in GLUT2 by doing site-directed mutagenesis and then expressing the mutant proteins in oocytes of *X. laevis*. We assessed their functionality using kinetics transport assays and tested their sensitivity to known inhibitors of GLUT2. Our results indicate that changing the polarity of the lateral chain of these residues causes a loss of function without affecting the K_M and the sensitivity to inhibitors, suggesting that these mutations affect the mechanics of the transporter rather than the interaction with the sugar. Our results support the notion that these residues are indeed crucial for the mechanics of sugar transport in GLUT2. We propose that they define a gate occluding the sugar binding-site from the extracellular environment.

103) PURIFICATION AND BIOPHYSICAL CHARACTERIZATION OF THE DNA-BINDING DOMAINS OF YEAST FORKHEAD BOX TRANSCRIPTION FACTORS

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Forkhead box proteins (Fox) constitute a family of transcription factors that are crucial for regulating several biological processes, from cell cycle timing to immune responses to language learning in different species. Recently, the DNA-binding forkhead domain from the FoxP subfamily of human transcription factors have been described to oligomerize via domain swapping (DS), a folding-upon-binding phenomenon leading to an intertwined dimer, which acts on gene regulation by physically mediating long-range chromosomal interactions. In *S. cerevisiae* there are four Fox homologues, FKH1, FKH2, FHL1 and HCM1. FHL1 and HCM1 regulate transcription of ribosome-activated proteins and S-phase-transcriptional activation, whereas FKH1 and FKH2 have been shown to control the timing of replication origins. Although a molecular mechanism for this phemonenon has not been established, DS has been proposed as a plausible way to enable bridging of two distal chromosomal loci. Consistently, sequence analysis has shown that substitution of a Fox-conserved proline by alanine (Pro-Ala), fundamental for enabling DS in FoxP, is also present in FKH1 and FKH2. Here, we characterized the aggregation state of the forkhead domains of all yeast Fox transcription factors as well as the equilibrium unfolding of FKH1 and FHL1. Size exclusion chromatography showed that all proteins are monomeric at high protein concentrations (>5 mg/ml), demonstrating that DS is not solely determined by the Pro-Ala substitution. However, equilibrium GdnHCl-induced unfolding showed that their stabilities vary from 1.5 (FKH1) to 3.0 kcal/mol (FHL1) regardless of their aggregation state, suggesting that this substitution destabilizes the forkhead fold, as seen for human FoxP1.

104) SIPP-POT: A NEW TOOL FOR CREATING AND EVALUATING DYNAMIC AND FULLY USER CONFIGURABLE STATISTICAL POTENTIALS BASED ON ATOM DISTANCES, BURIED AND ACCESSIBLE SOLVENT SURFACE AREAS FOR PROTEINS, NUCLEIC ACIDS, PROTEIN COMPLEXES AND PROTEIN-DNA COMPLEXES.

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The problem of evaluating the interaction between molecular structures is key in bioinformatics, due to its tremendous potential for enabling the prediction of the structure of modified or engineered molecules, accelerating research in many fields as varied as enzyme optimization and DNA binding site recognition. Condensing our lab's previous research, we have developed a tool that allows the user to generate and use statistical potential energy functions based on heavy atom interactions, which can be filtered by many features such as sequence topology at the residue and atom level, shielding angles and also based on our newly developed atom buried and accessible solvent surface area functions (BSA and SASA). We have benchmarked our tool's ability to generate potentials capable of predicting protein-DNA binding using a set of DNA binding proteins with known experimental dG binding data for various DNA sequences. Models of the complexes, free protein and free DNA were created with MODELLER for each DNA sequence with experimental dG values, which were then used in benchmarks for testing the accuracy in predicting ranking, indentifying native models from decoys, and measuring correlation to real dG values. Our potentials achieved a precision of 20% in predicting the experimental ranking in the set of protein-DNA complexes, an AUC of 0.91 in identifying the native structures among 330 non-native models and a correlation coefficient of 0.64 to the real dG values of 30 structures from a non-redundant set, which makes them compare favorably to existing protein-DNA potentials. Acknowledgements: Funded by FONDECYT REGULAR 1141172

105) CATHEPSIN L IS NECESSARY FOR SURVIVAL OF COLORECTAL CANCER CELLS UNDER METABOLIC STRESS

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Tumor cells can survive under metabolic stress, by evasion of programmed cell death or by promoting autophagy process. Some studies have shown that cathepsin L, a lysosomal protease is involved in these processes. Thus, we investigated whether cathepsin L is involved in cell survival of colorectal cancer cells exposed to metabolic stress. For this study, COLO32O and SW620 colorectal cancer cell lines were cultured in serum-free medium or medium without glucose to induce metabolic stress for 24 hours. Cathepsin L activity was inhibited by siRNA-mediated silencing or by drug inhibition. Subsequently, we analyzed the role of cathepsin L in cell viability, complemented with detection of specific markers of apoptosis and autophagy, and analysis of DNA integrity. To determine variations in the location and expression of cathepsin L, we performed immunofluorescence assays of cathepsin L expression, along with detection of cytoskeleton and lysosome markers. Furthermore, to analyze changes in cathepsin L activity, we used a specific substrate; this experiment was supplemented with analysis of the active forms of cathepsin L by zymography. Finally, to analyze changes on the expression of other lysosomal proteases after cathepsin L inhibition, we performed PCR analysis of cathepsin D and B. Our results showed that cathepsin L is necessary for colorectal cancer cells survival under metabolic stress. Cathepsin L inhibition leads to a defect in cell autophagy, which would be reflected in an increase in cellular apoptosis.

106) A NEW MODEL FOR PREDICTING DNA FLEXIBILITY FROM A NUCLEOTIDE SEQUENCE

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Nucleotide sequence determines DNA structural dynamics and thereby its flexibility, affecting DNA structure and protein-DNA recognition in biological processes such as gene expression or nucleosome formation. Data from experimental structures was used to generate a new computational model (pFlex) for predicting regions of higher/lower flexibility from a DNA sequence. 87 free duplex DNA structures from the Protein Data Bank (PDB) were used to calculate the structural dispersion (RMSD) for every possible dinucleotide base-pair superposition. Based on these results, a computer program assigns the expected RMSD value per nucleotide in the DNA sequence that constitutes a flexibility profile.

The results obtained for the comparison of this new method with six different dinucleotide and trinucleotide angle models previously published shows a high similarity with the tri-nucleotide model based on nucleosome positioning, which is the most robust and widely-accepted among all tested. Likewise, structural deviation for crystals of nucleosomes was calculated between the 3D structure and a B-DNA fiber model of the same sequence, and compared to pFlex, resulting in similar profiles and higher correlations than that observed for other models.

These shows that pFlex is able to accurately predict bending sites with less information (di-nucleotides) than a more complex and widely accepted model (tri-nucleotides). pFlex also predicts with high accuracy the observed DNA deformation from B-DNA conformation. This flexibility model could be implemented in the 3D modeling pipeline of duplex DNA, to facilitate the recognition of regions with more/less flexibility, which can be modeled and restraint with more/less degrees of freedom according to this information.

107) FLOCCULATION MEDIATED BY LIGHT: OPTOGENETIC CONTROL OF GENE EXPRESSION IN YEAST

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The budding yeast *Saccharomyces cerevisiae* is one of the most relevant microorganisms for industrial biotechnology. In yeast, flocculation is the cellular aggregation process controlled by *FLO* genes, a phenotype that is considered as an easy and low-cost method of yeast separation in industrial fermentation processes. Through the development of a simple synthetic optogenetic switch we have successfully implemented a blue-light responding transcriptional system to control gene expression and ultimately yeast flocculation. Therefore, now in yeast (which naturally does not respond to light) we can efficiently and orthogonally induce gene expression by adding such an optogenetic switch. The latter yields a broad dynamic range of expression, as evidenced by a luciferase reporter. The expression levels of this system were higher than the available commercial yeast inducible kits, based in chemical galactose activation. Moreover, we have assembled this optogenetic switch as part of a simple genetic circuit in order to control -at will- a relevant biotechnological process, such as flocculation. Thus, we activated flocculation by the presence of an external cue: light. Depending on how the circuit is assembled, we can trigger flocculation by the absence or presence of such stimulus. In conclusion, the optogenetic switch in yeast provides a versatile toolbox for biotechnological applications.

108) HMMTEACHER1.0 : A TEACHING RESOURCE FOR UNDERSTANDING HIDDEN MARKOV MODEL SOLUTIONS THROUGH PRACTICE.

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Hidden Markov Models (HMM) are one of the most powerful and obscure modeling techniques in Bioinformatic Sequence Analysis, in spite of - or maybe caused by - being theoretically well supported. Other applications of HMMs include speech recognition and topics in structural biology. Their use has increased in time in a shy way, in part because 1) its steep theory learning curve; 2) the need of good modeling and programming skills in order to implement HMM algorithms and 3) because HMMs have to be fed with large amounts of carefully curated data. In order to facilitate the use, understanding and practice of HMMs, we have developed a Java based software that solves HMM algorithms once the user inputs the model. The graphical interface guides the learner through five steps: Steps 1 to 3, requesting the main elements of a HMM; 4. Asking the user which one of the three questions (related to algorithms Forward, Backward and Viterbi) is to be answered and; 5. Reporting the solutions step-by-step in PDF format document. In the case the user doesn't know the information the software is requesting, it provides an option in which the program fills the unknown box with random generated numbers. This way, the student can go on and learn by examining the solution of the model. This first version allows the Bioinformatics teacher to focus the HMM classes on modeling the biology and in the problem solving practice instead of on the mathematical theory that leads to the algorithm formulae.

109) SUPPLEMENTATION WITH VEGETABLE OIL DECREASES OXIDATIVE STRESS PARAMETERS AND INFLAMMATION IN VISCERAL ADIPOSE TISSUE AND LIVER OF HIGH FAT DIET FED ANIMALS

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Rosa Mosqueta oil (RM, *Rosa rubiginosa*) contains alpha-linolenic acid, precursor of the omega-3 fatty acids eicosapentaenoic (EPA) and docosahexaenoic (DHA). EPA and DHA have anti-inflammatory and antioxidant properties; they stimulate beta oxidation and inhibit de novo lipogenesis, contributing to the improvement of insulin resistance (IR). EPA and DHA are protective agents against IR, which is directly related with hepatic steatosis and metabolic syndrome. Also, this oil is rich in tocopherols that would contribute to these effects. This work seeks to assess whether oral supplementation with RM oil decreases hepatic and adipose oxidative stress and inflammation parameters induced by a high fat diet (HFD). Male mice (n = 6-9 per group) C57BL/6J were fed for 12 weeks and divided into four groups: (i) control diet (20% protein, 70% carbohydrates, 10% fat); (ii) control diet and RM (1.94 mg ALA/g body weight/ day,COESAM ltda.); (iii) HFD (20% protein, 20% carbohydrates, 60% fat); (iv) HFD and RM. General parameters (body weight, visceral fat, histology), hepatic oxidative stress [TBARS (ELISA), Nrf2 (IHC), heme oxygenase-1 (WB) and carbonylated proteins] and inflammation [hepatic PPAR-alpha (IHC); hepatic, adipose and systemic TNF-α and IL-1β levels (qPCR, ELISA)] were evaluated. RM oil supplementation decreased (one-way ANOVA, Newman Keuls test, $P < 0.05$) oxidative stress and inflammation induced by HFD, along with recuperation of Nrf2, heme oxigenasa-1 and PPAR-α levels.In conclusion, supplementation with RM oil decreases hepatic oxidative stress by normalizing the levels of Nrf2, heme-oxygenase-1 and PPAR-α, and decreases adipose and systemic TNF-α and IL-1β levels, both effects associated with the prevention of steatosis.

110) NHE1 REGULATES INTRACELLULAR PH AND CELL PROLIFERATION IN HUMAN OVARIAN CANCER CELLS

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Ovarian cancer is one of the most lethal gynecological cancers. This disease is commonly detected in advanced stages, where is disseminated to the peritoneum and patients suffer malignant ascites. At cellular level, cancer cells presents elevated glucose metabolism, producing an increased amount of protons (H^+). Protons are extruded from the cytoplasm to the extracellular medium via different membrane transport systems including the Na+/H+ exchanger 1 (NHE1). NHE1 is one of the main mechanisms regulating the intracellular pH (pHi) involved in cell volume control, cell migration, and cell proliferation in several types of cancer. However, NHE1 expression and its role in human ovarian cancer is not yet described. In this study we show that NHE1 is expressed in human ovarian cancer. Functional studies show that NHE1 is one of the main pHi regulators after an acid-pulse in HOSE non-tumor, A2780 tumor cell line, and in primary cultures of ascites-derived (haOCCs) human ovarian cancer. In addition, the inhibition of NHE1 with zoniporide and hexametylenamiloride reduced cell proliferation in HOSE, A2780, and haOCCs. Expression of NHE1 in biopsies of human ovarian cancer correlates with ki67 (cell proliferation marker). Further, patients overall survival analysis (TCGA database) showed that amplification of the NHE1 coding gene *SLC9A1*, reduces overall patients survival. Altogether these findings suggest that NHE1 has a pro-proliferative role in human ovarian cancer. The increase in NHE1 activity may be an unfavorable prognostic factor in patients with this disease.

111) PDGF-BB INDUCES CHANGES IN MITOCHONDRIAL MASS AND FUNCTION ASSOCIATED WITH VSMC PHENOTYPIC SWITCHING

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The dedifferentiation of vascular smooth muscle cells (VSMC) from a contractile to a synthetic/proliferative phenotype, play a key role in the development of vascular pathologies like hypertension and atherosclerosis. This change involves metabolic alterations in VSMC. We propose that platelet-derived growth factor (PDGF-BB), a potent phenotype switching inductor, promotes changes in both quantity and function of mitochondria, and that these changes would be responsible of the observed metabolic alterations. VSMC A7r5 were treated with PDGF-BB (10 ng/mL) for 0-24 h. Mitochondrial function was observed measuring mitochondrial potential, cellular ATP content and oxygen consumption. Mitochondrial potential (Δψm) was evaluated with JC-1 staining by flow cytometry, cellular ATP content was measured using a luciferin/luciferase-based assay, and oxygen consumption was evaluated using a Clark Electrode. Mitochondrial mass was determined by qPCR and by measuring the levels of the mitochondrial proteins Hsp70 and MTCO using Western blot. Our results showed a decrease in both mitochondrial function and mass, and that this would be involved in the phenotypic switch of VSMC. Understanding the mechanism involved in the development of VSMC phenotype is critical for the future development of new treatments for vascular pathologies.

112) ML9 INDUCED-AUTOPHAGY CONTRIBUTES TO APOPTOSIS IN CULTURED CARDIOMYOCYTES

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ML9 (1-(5-chloronaphthalenesulfonyl)homopiperazine hydrochloride)) is a well known chemical inhibitor for STIM1-plasma membrane interactions that eventually prevents store-operated Ca^{2+} entry. Autophagy is a lysosome-mediated catabolic process of protein degradation, organelle turnover, and recycling of cytoplasmic constituents, often cytoprotective, serving to defend against disease-related stress and nutrient deficiency. Autophagic cell death in prostate cancer cells has been reported via downregulation of STIM1. However, no report is available that explain how ML9 mediated autophagy contributes to cardiomyocyte apoptosis. To investigate this, rat cardiomyocytes were cultured with DMEM/M199 media containing 2% FBS for 4 h. To assess the autophagy flux, bafilomycin A1 was used and LC3 processing was determined by Western blot. Cleaved caspase-3 as an apoptotic marker was detected by Western blot. Cell viability was assessed by Trypan blue exclusion test. Results showed that after 4 h of ML9 treatment LC3-I as well as total LC3 levels decreased significantly as compared to the basal levels. These changes persisted even when autophagic flux was blocked using bafilomycin A. No change in beclin-1 protein levels was observed. Incubation with ML9 for 4 h inhibited basal autophagic flux. Moreover, ML9 treatment decreased STIM1 protein levels in the presence or absence of bafilomycin A. Long treatments with ML9 resulted on the activation of caspase 3, indication of occurrence of apoptosis. These data suggest that ML9 inhibited autophagic flux, decreased STIM1 protein level and induced cardiomyocyte apoptosis.

113) ASSESSING THE EFFECT OF CALCIUM AND MAGNESIUM IONS IN THE STRUCTURAL STABILITY OF THE PROTEIN KINASE A THROUGH MOLECULAR DYNAMICS SIMULATIONS

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Protein Kinase A (PKA) has become the most well-known kinase due it was the first in being crystalized and characterized. Its role is to catalyze the transfer of a phosphoryl group from an ATP molecule to a peptide substrate. While Mg^{2+} is the preferred cofactor in kinases, it has been proven experimentally than other divalent metals such as Ca^{2+} can also promote the phosphoryl transfer but at much lower rates. Very recently, crystallographic and kinetic data for PKA have shown that in the presence of Ca^{2+} the stability of a peptide substrate is decreased while the same phosphorylated substrate would tend to be trapped in the active site. In order to get a better understanding of these events, MD and free binding energy simulations with the software Amber 14 and force field ff99SB using recent crystallographic structures for reactant and product states were performed. Our simulations could rationalize the main differences between Mg^{2+} and Ca^{2+} as PKA cofactors and the effect that Ca^{2+} hason the flexibility of the ATP molecule and other residues in the active site. Also, we could observe that Ca^{2+} tend to reduce the mobility of the phosphorylated substrate and the residues forming active site corroborating experimental observations about a trapping effect produced by Ca^{2+}. This information is expected to be valuable for the understanding of the mechanism in protein kinases which could lead to the design of more potent inhibitors as well as to understand a possible regulation mechanism exerted by Ca^{2+} on kinases.

114) ACTIVIDAD ANTIOXIDANTE DE DIFERENTES EXTRACTOS OBTENIDOS DESDE ESPECIES MICROALGALES.

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Las reacciones de oxidación asociadas a funciones propias de un organismo generan especies reactivas de oxígeno intracelulares (EROi) que se relacionan a daño oxidativo de distintas macromoléculas biológicas, desarrollo de diversas patologías, y envejecimiento. Los antioxidantes sintéticos son ampliamente cuestionados por sus efectos tóxicos y potencialmente cancerígenos, lo que ha derivado en la búsqueda de antioxidantes de reemplazo desde fuentes naturales como frutas, vegetales, macro y microalgas. El objetivo de este trabajo fue evaluar la capacidad antioxidante de 4 especies microalgales escasamente estudiadas, *Chlamydomonas reinhardtii, Nannochloropsis oculata*, *Scenedesmus obliquus* y *Tetraselmis suecica*, y compararlas respecto a *Arthrospira platensis* (nombre comercial Spirulina), conocida por sus propiedades antioxidantes. Extractos metanólicos, etanólicos y acuosos de estas microalgas fueron evaluados respecto a: 1) contenido de compuestos antioxidantes fenoles y flavonoides totales, 2) actividad antioxidante mediante tres métodos convencionales, $ABTS^{+}$, DPPH y FRAP, 3) capacidad para reducir la producción EROi en dos modelos de líneas celulares (SHK-1 y Hek293), 4) actividad citotóxica sobre estas líneas celulares, y 5) contenido de pigmentos ficobilínicos, carotenoides y clorofilas. *C. reindardtii*, seguida de *T. suecica* y *N. oculata* presentaron altos niveles de compuestos y actividad antioxidantes, así como capacidad de reducir la producción de EROi sin generar toxicidad celular. Estas especies constituyen fuentes potencialmente ricas en antioxidantes naturales, que podrían ser usadas a futuro para proteger contra la oxidación de productos de interés tanto para la industria alimenticia, cosmética u otras áreas en salud humana y/o animal.

115) STUDYING THE EFFECT OF CANCER MUTATIONS IN AGGREGATION-PRONE SUPERFAMILIES OF PROTEINS: AGGREGATION AS A PLAUSIBLE NEW MECHANISM OF ACTION FOR MUTATIONS IN CANCER

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(Sponsored by This Work Is Supported By FONDECYT De Iniciación Nº 11130578, Awarded To Daniel Almonacid)

Cancer is the main death causing disease in the developed world, and it is the result of genetic mutations, a large proportion of which affect protein-coding regions which may change the structure and thus, the function of the encoded protein. On the other hand, protein aggregation has been implicated in several neurological disorders like Alzheimer\'s, Lateral Amyotrophic Sclerosis, Parkinson's disease among others. The aggregation process is driven by aggregation prone regions (APR's) in proteins, which are mainly hydrophobic sequences present in more than 80% of the proteome. Based on recent evidence, we hypothesize that cancer mutations can trigger protein aggregation in aggregation-prone superfamilies of proteins, representing a novel mechanism of action for mutations in cancer. Gathering information from statistically significant mutated proteins in tumor exomes, protein superfamily databases, and databases of proteins that aggregate in disease, we identified 15 protein superfamilies significantly mutated in cancer which have also been implicated in diseases caused by protein aggregation. As a case study, we measured the structural impact from cancer mutations in the MHC type I proteins, specifically in beta-2-microglobulin (b2m). We found that particular point mutations in b2m affect the interactions between secondary structure elements in the protein, altering solubility, and bringing two known APRs close together. We plan to extend the use of this methodology to study mutations in other of the superfamilies identified in this work, to evaluate if this novel mechanism of action of mutations in cancer is universal, or specific to b2m.

116) COMPARATIVE TRANSCRIPTOMIC ANALYSIS OF THE DISTINCT PANCREATIC CELL TYPES AMONG DISTANT VERTEBRATE SPECIES

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Pancreas is a mixed gland composed of endocrine and exocrine tissues and plays a crucial role in the metabolism of all vertebrates. The endocrine cells are mainly grouped into the islets of Langerhans and secrete distinct hormones, such as glucagon (α-cell), insulin (β-cell), somatostatin (δ-cell) and ghrelin (ε-cell). Diabetes occurs when insulin production by the β-cells is unable to counteract increase of glycemia. The main goal of the present work is to determine the transcriptomic signatures of each pancreatic cell type in zebrafish in order to identify novel cell type-specific regulatory genes that might be crucial for their differentiation and physiology. Pancreatic acinar cells, ductal cells as well as the endocrine α-, β- and δ-cells were isolated from adult zebrafish using FACS and RNA-seq was performed from these cell types. Comparison between the RNA-seq datasets allowed us to highlight all genes with enriched expression in each cell type and to identify new markers of the mature pancreatic cells in zebrafish. In order to establish the expression blueprint of pancreatic endocrine and exocrine cells conserved from fish to mammals, we compared the pancreatic transcriptomes from zebrafish, mouse and human. Among the genes identified as conserved, we identified most of the transcription factors previously known to be important for pancreatic cell differentiation. This interspecies comparative analysis highlighted genes with evolutionary conserved expression whose pancreatic function is still unknown, but also revealed striking differences in gene expression patterns between species.

117) CHANGE IN E*NGRAULIS RINGENS* HATCHING ENZYME EXPRESSION TOWARDS SALINITY VARIATIONS.

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The Peruvian anchovy (*Engraulis ringens*), a pelagic species, is one of the important commercial fishes of Chile. It habits from Peru (6°S) to the Patagonia(47°S), a wide latitudinal range where environmental conditions vary markedly. Among these environmental conditions, the effect of variations in salinity has been studied, however, little is known about its effects on the marine organisms at the molecular level. In this study, anchovy eggs collected off Bahia de Coliumo (36º30`S) were incubated at different salinities, in order to study its effects on the hatching enzyme (HE) and genes that allows its expression, which by degradation of the chorion allows hatching. RT PCR measured the expression of the gene of the hatching protein. For quantification of the protein it was required the production of a polyclonal antibody, which allowed the detection of the protein by Western Blot. The hatching success was estimated by hatching rates. An increase expression of the gen HE was obtained in the lower experimentation incubating salinity (27psu), which is consistent with the results observed in the expression of the protein. The salinities where inhabits the anchovy (34psu) showed a lower HE expression. Thus, the change in salinity of the seawater in the area during the spawning period could affect gene expression of the HE and later protein expression. The results agree with field observations in the winter season when lower salinities and higher rates of hatching have been observed coinciding with the period of main spawning of this species in the area.

118) CHARACTERIZATION OF A MITOFUSIN-2 KNOCKDOWN SERTOLI CELL LINE.

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Sertoli cells have multiple roles in germ cell development, ranging from physical support to supply of nutrients. One of these functions corresponds to phagocytosis of residual bodies and apoptotic spermatogenic cells. The mechanisms that regulate Sertoli cell metabolism are central to the maintenance of spermatogenesis and male fertility. Despite being an energy substrate, glucose is not the main metabolite used for ATP synthesis in Sertoli cells, they preferentially use lipids as energy source. Mitochondrial quality control is a process that includes the exchange of mitochondrial components through mitochondrial fusion and fission and removal of the dysfunctional mitochondria through autophagy or mitophagy. Mitofusin 2 (Mfn2) is a mitochondrial outer membrane protein involved in the rearrangement of these organelles through the regulation of the fusion process. In this work we evaluated the role of Mfn2 in the maintenance of mitochondrial and phagocytic function in Sertoli cells. We generated a Sertoli 42GPA9 Mitofusin-2 knockdown (KD) cell line and evaluated different parameters of mitochondrial function and observed an 80% increase in ROS production, a 70% increase in mitochondrial membrane potential and a fragmented mitochondrial morphology, demonstrating an alteration in mitochondrial function. Phagocytosis was determined by incubating the cells with fluorescent beads and evaluated its internalization, which was reduced in a 30%. These results suggest a possible role of Mfn2 in Sertoli cell phagocytosis.

119) ROLE OF A3 ADENOSINE RECEPTOR ON THE STEMNESS OF GLIOBLASTOMA STEM-LIKE CELLS UNDER HYPOXIC CONDITIONS

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Introduction: Glioblastoma stem-like cells (GSCs) correspond to a cell subpopulation with stem cell characteristics within the glioblastoma. Given their tumorigenic capacity and greater radius and chemoresistance with respect to other cells in the tumor, the GSCs have been postulated as the main responsible for the failure of therapy. It has been observed that the hypoxic microenvironment is part of the GSCs niche, enhancing their resistance mechanisms and promoting stem phenotype (stemness). Under hypoxia, we have seen a marked increase in extracellular adenosine metabolism, which has been correlated with increased chemoresistance; however, the role of adenosine and its Adenosine Receptor A3 (A3AR) on the GSCs stemness not been explored. **Aim:** Determine the role of A3AR on the maintenance of stem phenotype under hypoxia. **Methodology:** Expression of CD133 and CD44 stem markers and GFAP differentiation marker, was measured by flow cytometry and western blot. The self-renewal capacity was evaluated by testing disaggregation/reformation of neurospheres. Chemosensitivity of GSCs was determined by MTT viability assays. All assays were developed in the presence of A3AR antagonist (MRS1220). **Results:** We observed a decrease in the CD133 and CD44 markers in hypoxic GSCs treated with MRS1220. These results were correlated with increased expression of GFAP and less capacity for self-renewal. The effect on the differentiation of GSCs treated MRS1220 was supported by an increase in the chemosensitivity to anticancer drugs. **Conclusions:** Through A3AR, hypoxic GSCs promote their stemness and increase their chemoresistance. The antagonism of adenosine signaling suggests an effective method to differentiate and chemosensitizing to GSCs.

120) DIFFERENTIAL CIS-REGULATORY ELEMENT IN PROMOTER OF DUPLICATED SOMATOLACTIN GENES IS RELATED TO ESTROGEN RESPONSE OF *SLβ* IN *CYPRINUS CARPIO*.

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Somatolactin (SL),a piscine hypophyseal hormone involved in background adaptation, reproduction and fatty acid metabolism which might be affected by estrogenic endocrine disruptor compounds. Two somatolactin genes were detected in pituitary of *Cyprinus carpio*. Only *slβ* but not *slα* responded with increased expression in pituitary of male adult carp to 17β-estrogen treatment respect to control as shown by RT-qPCR analyses. In addition, the proximal promoter region of *slα* and *β* was cloned and characterized. Indeed, in the proximal promoter of *slβ*, but not in *slα*, an estrogen response element (ERE) was predicted with Tfscan software and specific binding was confirmed *in vitro* EMSAs with pituitary nuclear extract. To clarify the role of this ERE in SLβ response to estrogen in a functional assay, two constructs were assembled, one containing wild type proximal slβ promoter and another with the same sequence but mutated ERE (EREmut) in front of firefly luciferase coding sequence. The assay was performed in rat pituitary cells GH3/BH6 cotransfected with a dual luciferase system and treated with 17β-estrogen. Clearly, wild type slβ promoter plasmid showed increased luciferase activity in response to estrogen correlating perfectly with the *in vivo* expression data. However, slβ promoter with EREmut plasmid showed no significant variation in luciferase activity, indicating that this particular ERE is related with the differential expression of *slβ* in response to 17β-estrogen. Taken together these data suggest that SL could serve as early indicator of neuroendocrine disrupting effects to assess biologically relevant changes in the aquatic environment.

121) REGULATION BY AMP IN BIFUNCTIONAL ADP-DEPENDENT SUGAR KINASE FROM *METHANOCOCCUS MARIPALUDIS*: KINETIC AND EVOLUTIONARY BASIS.

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In archaeal glycolysis, glucokinase and phosphofructokinase activities utilize ADP instead of ATP as phosphoryl donor. In organisms belonging to *Methanococcales* there is only one enzyme that perform both activities. Until now, all these enzymes have been reported as nonregulated. Recently, we described that the bifunctional enzyme from *Methanococcus maripaludis* (MmPFK/GK) is activated by their product AMP. However, the kinetic basis of this property as well as its evolutionary origin (ancestral trait or an evolutionary novelty) remains unknown. In this work we addressed the kinetic mechanism of the AMP mediated activation of MmPFK/GK and the evolutionary history of this trait by kinetic characterization of the extant enzyme and resurrected ancestors of *Methanococcales* and of other branches of this family of enzymes. We determine that both activities of MmPFK/GK are activated by AMP. Kinetic essays performed at saturating concentrations of MgADP suggest the existence of an allosteric site for the effector. Saturation curves for glucose at different AMP concentrations indicate that the activation occurs through an increment in sugar affinity without altering the maximum velocity of the reaction (83 % decrease in K_m and a K_A for AMP of 0.7 mM). Evolutionarily, this property was determined to be ancestral, as observed in characterization of resurrected ancestral enzymes. Apparently this trait goes along with bifunctionality, being present in the last common ancestor of bifunctional enzymes from *Methanococcales* and specific PFKs from *Thermococcales*, but absent in specific PFKs from *Thermococcales*.

122) TRANSCRIPTIONAL DYNAMICS IN THE CHILEAN FLOUNDER *PARALICHTHYS ADSPERSUS*, UNDER FASTING AND REFEEDING PROCESS.

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In the nature, the marine species face a number of changes in their environment to which they must necessarily adapt. In this context, the response of fishes to the food availability is complex and involves multiple factors that are mostly unknown. Compensatory growth (CG) is an accelerated growth phenomenon observed in animals upon re-alimentation following a period of dietary restriction. However, the fasting promotes changes in the metabolism and can produce oxidative stress. Moreover, the stress can promote the generation of reactive oxygen species (ROS) that are counteracted by antioxidant enzymes. However, the molecular mechanisms controlling this phenomenon still remain to be elucidated. In this study, we focus on Chilean flounder (*Paralichthys adspersus*), a native marine fish that undergoes compensatory growth. The molecular mechanisms regulating the muscle CG in Chilean flounder was evaluated by a nutritional restriction assay through RT-qPCR for the histone variants H2AZ, mH2A1 and mH2A2; and the antioxidant enzymes Catalase (CAT), Superoxide dismutase (SOD) and Glutathione peroxidase (gpx). The results demonstrated that during nutritional restriction all factors analyzed showed a differential expression concordant with an epigenetic response and a severe stress condition.. This suggests that epigenetic mechanisms can be deployed against stress generated by fasting. This new knowledge may be relevant to the improvement of aquaculture techniques.

123) LIPOTOXIC STRESS-DEPENDENT E3 LIGASE MUL1 REGULATES CARDIAC MITOCHONDRIAL DYNAMICS AND INSULIN SIGNALING

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Introduction. Saturated fatty acids are among the dietary factors that cause severe cardiovascular perturbations. This lipotoxic stress promotes mitochondrial dysfunction and metabolic disturbances such as insulin resistance. The E3 mitochondrial ligase Mul1 stimulates mitochondrial fission and negatively regulates insulin signaling. **Methodology**. To establish a relationship between the adverse effects of lipotoxic stress in heart and Mul1, we set up an *in vitro* model of lipotoxicity, culturing cardiomyocytes with the saturated fatty acids myristic (MA) or palmitic acid (PA). *In vivo* model of diet-induced obesity (mice fed with high fat diet (HFD)), was also used. **Results**. Cardiomyocytes treated with MA developed hypertrophy and insulin desensitization. Also, PA impaired insulin signaling and decrease Akt activation. Furthermore, MA triggers mitochondrial fission in cultured cardiomyocytes and decreases the levels of mitochondrial fusion protein Mfn2. In this context, MA and PA increased Mul1 protein levels. In cardiac protein extracts from HFD model, Mul1 levels were also increased with a concomitant decrease in Mfn2 levels. **Conclusion:** These data suggest that Mul1 could mediate cardiac lipotoxic stress.

124) AUTOPHAGY ACTIVATION IN THE HEAD KIDNEY OF THE FINE FLOUNDER (*PARALICHTHYS ADSPERSUS*) UNDER CONFINEMENT STRESS

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(Sponsored by Supported By FONDAP 15110027 And FONDECYT 1130545)

Introduction: In mammals, stress can trigger autophagy, a route that is fundamental to control the levels of non-functional protein aggregates and dysfunctional organelles. In contrast, little is known about autophagy triggered by a stress condition in lower vertebrates. In this context, the aim of this study was to determine if the stress induced by a high stocking density is able to promote autophagy in fine flounder.

Methodology: Juveniles fine flounder were subjected to high stocking densities to induce a chronic stress condition. After 4 and 7 weeks, head kidney samples were collected from control and treated fish. Stress related genes (*gr1, gr2, mr, hsp70, klf15 and redd1*) and autophagy markers (*bnip3, lc3* and *atg14*) were assessed by qPCR. Moreover, autophagy flux (SQSTM1 and LC3 I-II) was evaluated by *Western blot*.

Results: Beside all stress related genes were significantly up-regulated after 7-weeks, no changes were observed at 4-weeks of treatment. Protein and mRNA levels of autophagy markers were also up regulated at 7-weeks. LC3-II protein content was increased and *lc3*, *atg14* and *bnip3* RNA levels were increased.

Conclusions: It was demonstrated that high stocking density triggers a strong stress response in the head kidney of the fine flounder, and at long-term, also activate autophagy. Further analyses are required to determinate how stress-induced autophagy contributes to recover kidney cellular homeostasis in fish.

125) LOX-1 RECEPTOR IS ESSENTIAL IN DIFFERENTIATION AND PRESERVATION OF PRO-FIBROTIC PHENOTYPE OF CARDIAC MYOFIBROBLASTS

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Background. Infarct myocardial (IM) triggers differentiation of cardiac fibroblasts (CF) to myofibroblasts (MCF). MFC perpetuates and secrete high levels of extracellular matrix components, which generates a long-term cardiac fibrosis that culminate in arrhythmias, heart failure and failure of the organ. The oxidized low density lipoprotein (oxLDL) is an important cardiovascular risk factor. Its mechanism of action is mediated by LOX-1 receptor. Subsequent to IM, oxLDLs are elevated in the area of damage. In LOX-1 knockout mice subjected to ischemia/reperfusion, there was a demonstrated increase in survival and decreased in collagen type I (typical marker for fibrosis). Preliminary results from our laboratory indicate that there is an increase in the expression of LOX-1 during the differentiation of the MFC. **Objective.** Determine whether the LOX-1 receptor is essential in differentiation of CF to MCF, and if oxLDL increases the pro-fibrotic phenotype in MFC. **Results.** In silenced CFs with siRNA for LOX-1: a) the expression of LOX-1 decreased 50% during the differentiation process of CF to MCF; b) the final phenotype expressed low levels of α-SMA (marker of MCF) and COL-1; c) Morphology observed by fluorescence microscopy did not correspond to MCF, compared to its control. MCF treatment with oxLDL for 72 hours maintained the levels of α-SMA and increased the levels of COL-1 and LOX-1. **Conclusion.** LOX-1 is essential for differentiation of CF and treatment of MFC with oxLDL increases the fibrotic phenotype.

126) EVOLUTIONARY CONSTRAINTS DETERMINE THE THREE-DIMENSIONAL DOMAIN SWAPPING OF THE FORKHEAD DOMAIN OF FOXP TRANSCRIPTION FACTORS

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The forkhead box (Fox) proteins are a widespread family of transcription factors, having 50 members only in humans. While most DNA-binding domains of Fox proteins exist as monomers in solution, recent structures from members of the P subfamily (FoxP1-4) show that they can form dimers via three-dimensional domain swapping (DS), a mechanism where the exchange of identical segments between subunits leads to intertwined dimers stabilized by intermolecular interactions similar to those observed in the isolated monomer. Recent experimental evidence suggests that FoxP1 undergoes rapid DS through localized structural rearrangements rather than needing extensive unfolding, in contrast to the established consensus about the DS mechanism. In order to understand how DS emerged in the FoxP subfamily, we computationally analyzed the evolution of these proteins. Phylogenetic analysis of human Fox proteins and ancestral sequence reconstruction shows that FoxP and FoxO diverged from a common ancestor (ancOP) that retains a conserved motif for all monomeric Fox family members (FPYF). In contrast, the common ancestor of FoxPs (ancP) shows a substitution of proline by alanine that has been experimentally described as required to enable DS. Finally, simulations using structure-based models with symmetrized interactions, commonly employed to explore DS in other proteins, did not reach the expected intertwined dimer. Yet, enriching these models with coevolutionary-based intermolecular interactions, extracted from multiple sequence alignments of FAYF-containing proteins, using mean-field direct coupling analysis, led to formation of the DS dimer up to 75%. These results suggest that evolutionary constraints sculpted the DS mechanism in the FoxP subfamily.

127) A_1 ANTAGONIST DPCPX BLOCKS INSULIN-INDUCED GLUCOSE UPTAKE INDEPENDENT OF INSULIN RECEPTOR ACTIVATION IN ADULT CARDIOMYOCYTES

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Adenosine is a key paracrine/autocrine signaling molecule that regulates tissue homeostasis both in physiological and pathophysiological conditions by activating four G protein-coupled adenosine receptors (ARs), A_1, A_{2A}, A_{2B}, and A_3 which are widely expressed in the body. A growing body of recent evidence suggests that adenosine participates in the control of insulin signaling in liver, muscle, pancreas and adipose tissue. However, despite of ARs expression have been reported in cardiac cells such as coronary artery endothelial cells, fibroblasts and cardiomyocytes a potential cross-talk between adenosine and insulin signaling has not be addressed in the heart. In the present study, we selected a set of frequently used standard AR ligands (4 agonists and 4 antagonists) and investigated their role in the glucose uptake induced by insulin in adult cardiomyocytes. Our results showed that A_1 antagonist DPCPX blocked insulin-induced glucose uptake independent of insulin receptor activation in adult cardiomyocytes, strongly suggesting that AR_1/Akt signaling could be associated with insulin resistance in diabetic cardiomyopathy.

128) NEW MICRORNAS IDENTIFIED THROUGH MICROARRAY AS REGULATORS OF BRCA1 EXPRESSION IN BREAST CANCER.

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BRCA1 is a tumor suppressor gene which mutations confer a high risk to breast cancer. We have previously described that 50% of hereditary breast cancer tumors with no germline mutation lose BRCA1 expression. In the case of sporadic breast tumors 30% do not show BRCA1 expression. Different somatic events silencing BRCA1 expression have been described in breast cancer tumors, such as promoter hypermethylation and BRCA1 gene deletion. These mechanisms do not completely explain BRCA1 loss of expression in breast tumors. A relevant mechanism in silencing gene expression is the action of miRNAs, extensively described in different types of cancer up to now. Our aim is to identify miRNAs differentially expressed in tumors with negative/moderate BRCA1 protein, which could regulate BRCA1 expression. We isolated total RNA from 50 fresh frozen breast cancer tumors, and miRNA profiling was performed using Human miRNA Microarrays (Agilent Technologies). Sixteen miRNAs were found exclusively upregulated in BRCA1-negative, and ten in BRCA1-moderate tumors ($p<0.05$). In silico analyses predicted eleven miRNAs that could regulate BRCA1 expression. We assessed the effect of 5 of these microRNAs using a Luciferase reporter assay in HEK293 cells. Briefly, cells were transfected with a pmiR-report plasmid containing the BRCA1 3'UTR, or a segment of its coding region, within the 3' end of the Luciferase reporter gene. Cells were co-transfected with microRNA mimics for each of the selected microRNAs. Additionally, endogenous BRCA1 expression was evaluated upon transfection of MCF10A cells with each microRNA mimic.

129) HANDLING STRESS STIMULATES TELEOST MITOCHONDRIAL BIOGENESIS SPECIFICALLY IN SKELETAL MUSCLE

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Introduction: In mammals, it has been described that increased levels of cortisol during stress are fundamental for mediating hypermetabolic conditions. This situation is characterized by accelerated protein catabolism in skeletal muscle, a stimulated gluconeogenesis in the liver, and increased mitochondrial biogenesis specific for skeletal muscle. Thus, the aim of this research is to study the differential expression of genes involved in mitochondrial biogenesis in red-cusk eel (*Genypterus chilensis*) skeletal muscle and liver, under handling stress. **Methodology:** Through *in vivo* approached we assessed the effects of stress mediated by cortisol on gene expression in skeletal muscle and liver of molecules involved mitochondrial biogenesis such as: 5' AMP-activated protein kinase *(ampk*), peroxisome proliferator-activated receptor gamma (*pgc1α*), sirtuin 3 (*sirt3*), among others. **Results:** Stressed fish only showed significant differences in mRNA and protein contents of *pgc1α* and *sirt3* in skeletal muscle, not in liver. Amounts of carbonylated proteins, lipid peroxidation and DNA oxidative damage induced by stress did not show changes between skeletal muscle and liver. **Conclusions:** Our results suggest that handling stress promotes skeletal muscle mitochondrial biogenesis, suggesting an elevated resting metabolic rate during stress.

130) REGULATION OF AUTOPHAGY BY POLYCYSTIN-2/BECLIN 1 COMPLEX

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Introduction: Autophagy is an intracellular recycling process in which macromolecules are sequestered and degraded by autophagolysosomes. One of the major regulators of autophagy is Beclin 1 (BCN1), a bridge-protein between the class-III phosphatidyl-3phosphate kinase and several modulators of its activity. Polycystin-2 (PC2) is a Ca^{2+} channel involved on the control of the mTOR pathway, a classic autophagic inhibitor. Recent studies have shown that starvation, a known autophagy inducer, re-localizes BCN1 to the same intracellular places where PC2 has been reported to be. Moreover, PC2 and BCN1 share a coiled-coil (CC) domain, in both cases necessary to interact with other proteins. We propose that PC2 is required for autophagy, and that the induction of autophagy is regulated by the PC2/BCN1 complex.

Material and Methods: Experiments were performed in HeLa cells. Specific siRNAs were used for down-regulate PC2. PC2 was overexpressed with AdPC2. For PC2 mutant transfection, plasmids (2µg/24h) were used. Co-immunoprecipitation was performed with a TBS+NP40 buffer. Data are shown as mean ± SEM.

Results: We showed that PC2 is required for basal and rapamycin-induced autophagy. Additionally, PC2-overexpression drives mTOR-independent autophagy increase. Our data also showed that PC2 and BCN1 form a complex that is modulated by starvation. Finally, our results suggest that EF+CC1 domain of PC2 is required to interact with BCN1 and induce autophagy.

Conclusions: Our results suggest that PC2 regulates autophagy downstream mTOR by forming a complex with BCN1. Therefore, pathologies with autophagy and mTOR deregulation, such as muscle atrophy, could be reestablished by modulating PC2-BCN1 complex formation.

131) EFFCT OF HIGH STOCKING DENSITY STRESS ON mRNA LEVELS OF MARKER GENES FOR GROWTH AND HEALTH IN SALMO SALAR

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High stocking density in aquaculture, where fish maybe exposed to constant overcrowding, chronic stress may affect diverse physiological mechanisms, resulting in detrimental effects on health and productivity. Quantifying mRNA levels of marker genes allows the early detection of stressors in the aquatic environment.

In order to evaluate the expression profile of marker genes involved in growth and health, juvenile *Salmo salar* were maintained at 10 kg/m^3 (control) or 70 kg/m^3 (high density). Hypothalamus, pituitary and liver was analysed by RT-qPCR in 4 individuals, collected at 1, 3 or 15 days from control and high density condition.

Fish kept at high density for 15 days showed an altered expression profile of genes involved in metal homeostasis, metallothionein mt and metal transcription factor mtf-1, of all evaluated organs. After 3 days of high stocking density, cytochrome P450-1A mRNA levels increased significantly in liver. In pituitary gland, for the first time elevated mRNA levels of somatolactin (sl) were detected in response to stocking stress, where the two isoforms reacted differentially, slβ increased at day three (3.6 times) and fifteen (10 times) respect to control, whereas slα was higher only at day 15 (10 times). Furthermore, gh mRNA levels were increased (2.6 times) after 15 days in high density. These results reflect that the stress response to high density could involve modulation of growth and detoxifying pathways, indicating that the response is complex and therefore, early identification of stress reaction could be critical for fish health in farming industries.

132) EGGSHELL MEMBRANE AS A SUPPORT FOR ENZYME IMMOBILIZATION

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The challenge of increasing both enzyme availability and enzyme stability may be addressed through enzyme immobilization, which provides an excellent base for increasing availability of enzyme to the substrate with greater turnover over extended times. Currently, immobilized enzymes are preferred over their free counterpart due to their prolonged availability that curtails redundant downstream and purification processes. Avian eggshell are biomineralizing systems extensively represented in nature. From a microscopic structural view, chicken eggshell is a highly ordered array of multiple layers of fibrillary membranes, The innermost layer of an eggshell is composed of two non-mineralized sublayers, the so-called inner and outer shell membranes structured by collagens (types I, V and X) and other proteins and glycoproteins containing lysine-derived cross-links. Mild conditions (i.e. dilute acetic acid treatment) are enough to separate the membranes from the calcified layer. In the present work we prepared glutaraldehyde-derivatized eggshell membrane, which was used to immobilize β-galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23). Comparison of kinetic parameters and enzyme properties for bound and free enzyme shows remarkable similarity. Structural information available for the enzyme supports our observations. Thus, we believe eggshell membrane is an appropriate support for immobilizing enzymes suggesting a promising avenue of applications, for example, in the food industry

JLA and EKC FONDECYT 1150681; VCF FONDECYT (Postdoc) 3160332

133) HYDROGEN PEROXIDE OR LEPTIN PRIMING INDUCES TRANSCRIPTIONAL MEMORY AT HMOX-1 GENE IN ENDOTHELIAL AND EA.HY926 CELLS.

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Introduction: Obesity during pregnancy increases probability to get Large for gestational Age (LGA) fetuses; also, is related to oxidative stress markers elevated levels in both maternal peripheral blood and fetus cord blood. Chorionic and umbilical arteries from LGA fetuses show endothelial dysfunction associated to an altered response to oxidative stressors. We proposed that oxidative stress can induce a programming in the manner of response to stressors in human artery endothelial cells (HUAEC) or EA.hy926 endothelial-like cells. **Methodology:** RT_qPCR: mRNA relative quantity. pHyper-cyto vector: antioxidant cellular capacity. **Results:** 1.- H_2O_2 Doses versus Response curve in HUAEC isolated from LGA fetuses showed a doubling EC50 in comparison to AGA cells (EC50= 104,9 µM for LGA ; 44,89 µM for AGA); HUAEC-LGA showed altered Nrf2 and Hmox-1 mRNA basal level; and, under H_2O_2 stimulation, induced an Nrf2 increased transcription. 2.- Using H_2O_2-Double-Hit protocol in HAUEC-AGA cells only Hmox-1 mRNA showed an Transcriptional Memory-like response. 3.- In EA.hy926 cells modified H_2O_2-Double-Hit protocol gave transcriptional memory-like response at 3 hours of second hit in Nrf2 and Hmox-1 mRNAs level. 4.- Leptin hormone induces Hmox-1 mRNA expression after 24 hours of 100 ng/mL treatment in EA.hy926 cells. 5.- Leptin-Priming allowed an Hmox-1 and Nqo1 increased transcription in an transcriptional memory manner after 3 hours tBHP-hit; and Nqo1 and Nrf2 mRNA, after 3 hours tBHQ-hit. **Conclusions:** H_2O_2 or Leptin Priming can induce a change in Hmox-1, Nrf2 and Nqo1 gene expression in response to stressors, indicating that probably epigenetics mechanisms are underling endothelial dysfunction in LGA arteries

134) OXLDL/LOX1 PATHWAY ACTIVATION: EFFECT ON CARDIAC MYOFIBROBLAST AUTOPHAGY AND DIFFERENTIATION

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Introduction: Oxidized low density lipoprotein (oxLDL) levels are elevated in chronic inflammation and is associated with the development of cardiac fibrosis. Cardiac myofibroblasts (CMF), the major responsible of cardiac fibrosis, express high levels of oxLDL receptor, LOX1. Results from our laboratory showed that basal autophagy is higher in MFC than fibroblasts. However, effects of oxLDL on fibroblast/CMF differentiation and autophagy are not known.

Objective: To study the effect of oxLDL on CMF autophagy and differentiation *in vitro*.

Methods: Primary adult rat CMF culture was stimulated with nLDL (naive LDL) or oxLDL. LOX1, αSMA (differentiation marker), NADPH oxidase2 (NOX2; downstream element of oxLDL/LOX1 pathway), LC3II and p62 (autophagy markers) protein levels were determined by Western blot. Cell viability and ROS production were determined by flow cytometry. CMF phenotype was assessed by immunofluorescence. LOX1 expression was downregulated using siRNA.

Results: oxLDL (20 ug/mL) induced CMF death at 24 hours. oxLDL triggered ROS production and NOX2 activation (as verified by p47 phosphorylation)**,** and increased LOX1 and αSMA protein levels. Pretreatment with LOX1 siRNA inhibited oxLDLinduced αSMA increase. oxLDL did not change the autophagic markers LC3II and p62.

Conclusions: oxLDL/LOX1 pathway may play a key role on fibroblast/CMF differentiation, suggesting a novel pharmacological target for cardiac fibrosis treatment. oxLDL does not trigger autophagy in CMF.

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85) GENETIC CHARACTERIZATION OF *TRYPANOSOMA CRUZI* IN CHRONIC CHAGASIC PATIENTS TREATED WITH NIFURTIMOX

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Introduction: Chagas disease (ChD), caused by the flagellated protozoan Trypanosoma cruzi is an endemic and important problem of public health in Latin America. Eventhough Chile has been declared free of transmission of T. cruzi by Triatoma infestans since 1999, it is estimated that there are over 150.000 individuals infected from Arica and Parinacota (XV Region, Latitude: -18° 28' 17.99" S) to the O'Higgins (VI Region, Latitude: -34° 10' 1.20" S). According to the nomenclature used in recent years, T. cruzi is clasfied into six distinct discrete typing units (DTUs) named as TcI-TcVI, with a seventh one limited only to bats (TcBat). This study focused on aimed the molecular identification and genotyping of T. cruzi in 30 Chilean chronic chagasic patients (ECh) from Coquimbo (IV Region, Latitude: -29° 57' 11.95"S) that have been treated with nifurtimox (NFX).

Methodology: NFX was administered under Informed Consent during the years 2009-2010 for two months. DNA extractions (pre and post therapy) were prepared from blood samples received in Guanidine/EDTA. The presence of T. cruzi was determined by qualitative PCR using primers specific to amplify the minicircle variable region of the kinetoplastid DNA (kDNA) and the highly repetitive nuclear satellite DNA (satDNA). T. cruzi genotyping was performed by amplification of miniexon and 18S and 24Sα rRNA genes.

Results and Conclusions: The genotyping performed in 30 cases evidenced that infections with T. cruzi-DTUs corresponding to TcII/TcV/TcVI group. Genotyping methodology can be performed by conventional PCR in blood samples of patients with low parasitemia

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