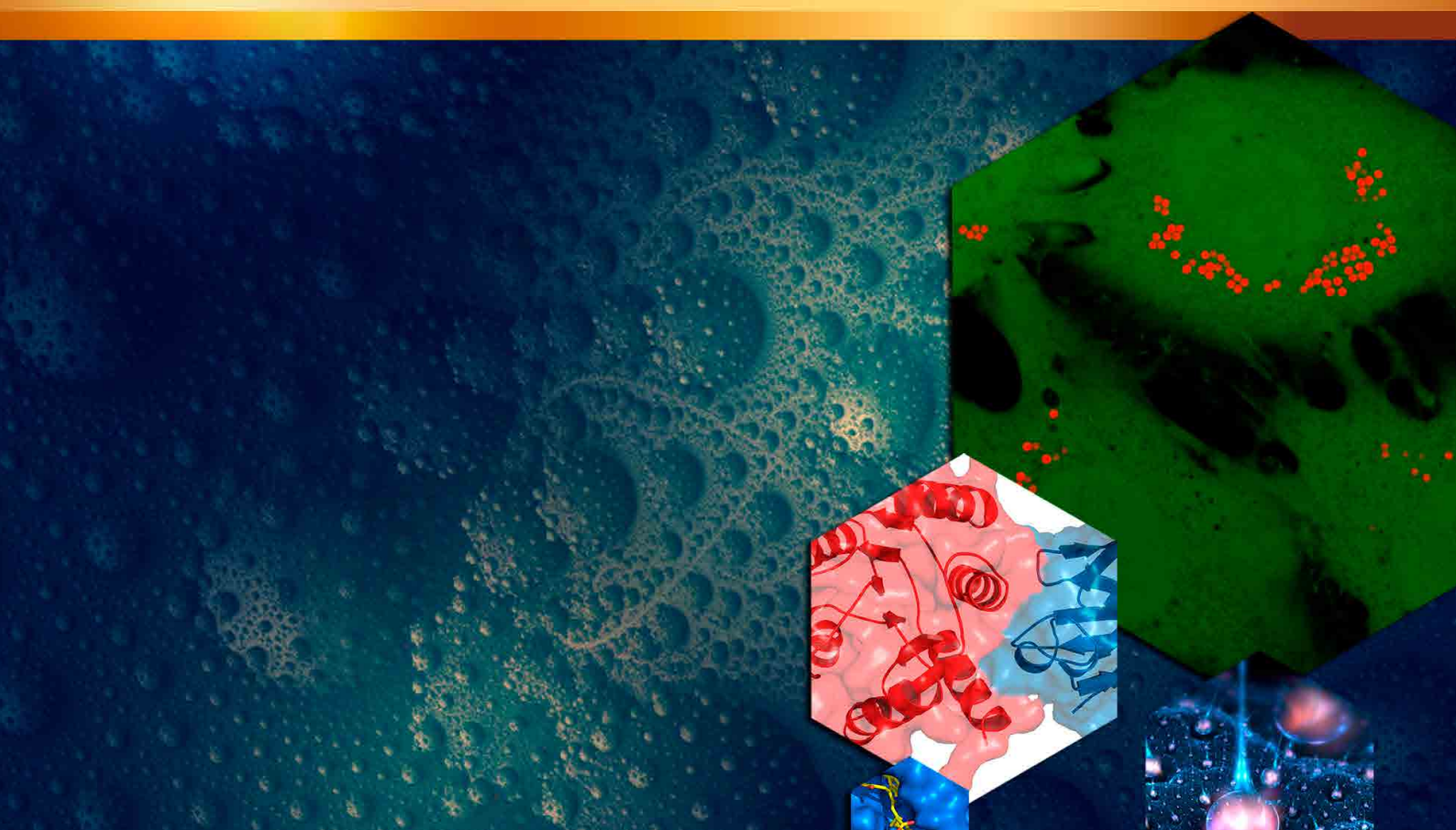
XL Annual Meeting

Chilean Biochemistry and Molecular Biology Society

September 26 to 29, 2017, Puerto Varas.

40 Years Contributing to Research in Chile: Looking Back & Moving Forward



XL Reunión Anual SBBMCH
26-29 de Septiembre 2017, Puerto Varas

FALLING IN LOVE WITH SCIENCE ... A WAY OF LIFE

The Chilean Society for Biochemistry and Molecular Biology promotes theoretical and experimental research, leading to the advancement and dissemination of Biochemistry and Molecular Biology in Chile. The Society also encourages and promotes initiatives aimed at maximizing the use of science for the benefit of the country and its citizens. This dream was founded in 1974, when Dr Hermann Niemeyer, Dr Marco Perreta, Dr Lylian Clark, Dr Enrique Beytia, Dr Arnaldo Foradori and Dr Arturo Yudelevich signed the document that gave legal status to the Chilean Society for Biochemistry and Molecular Biology. Subsequently, the first Annual Meeting was held in 1977.

This year, our society celebrates the XL version of its Annual Meeting. Despite the passage of time, the quality, excellence and love for science remain the central axis of each and every one of our Meetings. Currently, the Society has more than 150 members, including researchers from Chilean and foreign universities. The enthusiasm and dedication to scientific activities of all our members constitute the body and soul of this Society. Our Annual Meeting aims to be an open door to the scientific community, at national and international levels, and a showcase for scientific excellence. We hope that the colleagues who entrusted us with the dissemination of their work at our meeting, can appreciate our firm commitment to high-quality research, with the solid aim of pursuing the advancement of biochemistry and molecular biology. As the Directive of the Society, we wish to transmit a strong signal that each member is important to us. The previous Directive began the process of legalizing the Society, a process that is culminating with the update of our statutes in the current Directive. It has been an honor for us to serve you and the Society in this exciting and historical process. We also believe in decentralization, for which we have been holding regional meetings in different cities throughout the country represented by a local Director, in addition to supporting several initiatives proposed by members.

We wish to conclude this editorial letter by thanking all the people and institutions who, by placing their trust in this Directive, together with you, have made it possible to transform the dream of the founding colleagues of our beloved Society into a reality. To all our partners and friends of the Society, we say “WELCOME” and we hope you enjoy this excellent Annual Meeting that gathers prestigious national and international researchers. We also invite you to help us in the continuous growth of your Society, the Chilean Society for Biochemistry and Molecular Biology.

Ilona I. Concha, President; Luis F. Larrondo, Vicepresident; Marcelo López-Lastra, Past-President; Christian A.M. Wilson, Secretary; Patricio Ramos, Treasurer; Claudia Stange, Director for Santiago; Lorena García, Director for Santiago; Luis Morales, Director for Talca; Maximiliano Figueroa, Director for Concepción; Claudia Quezada, Director for Valdivia.

XL Annual Meeting
Sociedad de Bioquímica y Biología Molecular de Chile

September , 2017
Puerto Varas

DIRECTORY

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LECTURES

Opening Lecture

Entry of enveloped viruses into cells and eukaryotic cell-cell fusion processes: a common origin dating back to LECA?

Rey F[1], [1]Département de Virologie, UMR 3569 CNRS Institut Pasteur.

Enveloped viruses infect cells by inducing the fusion of viral and cellular membranes for entry. Structural studies have identified three structural classes of viral fusion proteins (I, II and III), which undergo a fusogenic conformational change upon interactions with the cell to catalise the membrane fusion reaction. Although the structures are totally unrelated, the catalytic mechanism appears to be the same for the proteins of the three classes: insertion of a non-polar segment of the protein into the target membrane to make a trimeric, extended intermediate, followed by fold-back of the rod-like intermediate into a hairpin. This re-arrangement brings both membrane-interacting segments of the protein to the same end of a very stable, post-fusion trimer, thereby drawing the two membranes together. Our previous studies have identified that the viral fusion proteins belonging to class II, which are characterized by the presence of thee beta-sheet-rich domains, are homologous to proteins responsible for somatic cell fusion in eukaryotes. We have now identified that they are also homologous to the ancestral gamete fusion protein HAP2, present in the main eukaryotic branches except for fungi, and proposed to have been probably present in the last eukaryotic common ancestor (LECA). This discovery supports the notion that gene exchanges between viruses and cells have had a strong impact in evolution, and furthermore points to a possible role of viral envelope genes in the emergence of sexual life on earth. I will discuss the broad implications of these recent findings in my talk.

Acknowledgements: ERC Advanced grant project (340371) CelCelFus; Grant ANR-2010-BLAN-1211 01; reccuring funding from CNRS and Institut Pasteur.

Felix Rey is a structural biologist who obtained his PhD in 1988 at Paris XI, and then went for a 7-years post-doctoral period at Harvard University, until 1995, where he specialized in the structure of viruses. He became a Junior PI in 1995, working at CNRS in Gif-sur-Yvette (Paris area), where in 1999 he became Director of the CNRS Laboratory of Structural and Molecular Virology. In 2004, he joined Institut Pasteur as Head of the Virology Department (which he directed until 2012), and in parallel he launched the Structural Virology Unit, which he still directs today. His research focus has included the 3D structures of viral polymerases (like the hepatitis C virus RNA-dependent RNA polymerase) and the nucleoprotein template of replication for negative stranded RNA viruses such as the respiratory syncytial virus. But his main focus has been the study of viral envelope proteins, how they induce fusion, and how they are recognized by potently neutralizing antibodies. His studies in this area have very important implications in “reverse vaccinology”, open the way for epitope-focused vaccine design for viruses such as dengue and Zika. Felix Rey is an EMBO member since 2005 and a member of the French Academy of Sciences since 2010. Among the honors and awards he obtained are the CNRS “Médaille d’Argent” in 2004, the Beijerink Virology Prize of the Dutch Royal Academy in 2013 and the Pasteur-Weizmann-Servier award in 2015.

Osvaldo Cori Lecture

Bacterial division depends on FtsZ GTPase activity as a motor of filaments treadmilling, a conserved evolutionary tubulin family mechanism.

Monasterio O[1], Araya G[1], Montecinos F[2], Diaz C[3], Lagos R[1], [1]Departamento de Biologia, Facultad de Ciencias, Universidad De Chile.[2]Eunice Kennedy Shriver National Institute of Child Health and Human National Institute of Health.[3]Howard Hughes Medical Institute University of California.

FtsZ and BtubAB are bacterial tubulin family proteins, important for cytokinesis and shape of bacteria respectively. FtsZ filaments showed treadmilling associated to GTPase activity. This work assessed experimentally the hypothesis that FtsZ self-assembles in the presence of GDP and GTP and that a conformational change occurs in FtsZ structure when these nucleotides exchange. The conformational changes induced by exchange between GDP and GTP nucleotides, was characterized using the intrinsic fluorescence of site directed tryptophan mutants located at the N-domain (F40W), the interdomain interface (Y222W) and the C-domain (F275W). Tryptophan lifetimes were measured using time-resolved fluorescence, knowing that the excited state is sensitive to changes in local environment, and changes at the interdomain interface were detected upon nucleotide exchange. These conformational changes were related to a hinge-like movement between FtsZ domains that might be responsible for the transition between straight and curved polymers. To understand the evolutionary importance of treadmilling as a key process for the dynamics of prokaryotic cellular cytoskeleton we determine the dynamics of *Prosthecobacter* BtubA/B. The results showed that BtubA/B assembly was consistent with a nucleation-elongation mechanism of polar bacterial microtubules that induced GTPase activity and, individual bacterial microtubules displayed treadmilling, which is characterized by the growth at one end and the shortening at the other end at the same rate. We conclude that treadmilling is a conserved mechanism of tubulin protein family that required GTP hydrolysis and a conformational change for its cellular function.
FONDECYT Project 1130711

Dr. Octavio Monasterio obtained the professional title of Biochemist (1971) at the University of Chile after studying for five years at University of Concepción. His undergraduate thesis was on initiation of protein translation and was directed by Dr. Jorge E. Allende. He started his academic carrier at the Department of Biochemistry, University of Concepción (1972) and in 1977 he moved to the Faculty of Medicine, University of Chile, to finally move in 1980 to the Faculty of Sciences, University of Chile where he has been until now. He obtained his Doctoral Degree at the University of Chile (1980) working on the kinetics mechanism of glucokinase under the direction of Dr. Hermann Niemeyer F. From 1980 to 1984 he was a Research Associate at Brandeis University, Boston, USA, in the laboratories of Dr. Sergei Timasheff (Protein Biophysics) and Alfred Redfield (Nuclear Magnetic Resonance). From 1986 until now his research was continuously funded by FONDECYT. In 1993 he was awarded with a grant from FONDEF for the creation of the "Centro de Equipo Mayor" with its own building. This Center has been successfully working for the support of academic and productive activities. In 2009 he was part of an European project for four years, as the only non European participant, in an International Consortium denominated DIVINOCELL, for the search of new antibacterial molecules. His present research is aimed to understand the molecular basis of the relationship between structure and function of tubulins and FtsZ proteins as components of the eukaryotic and prokaryotic cytoskeleton, respectively. The knowledge of molecular bases of recognition between FtsZ, ligands and other proteins of the bacterial *divisome* is crucial for the design of new inhibitors of tubulin and FtsZ polymerization, like future antitumor drugs and bacterial antibiotics, respectively. With other scientists he founded the first undergraduate career of biotechnology in our country, called "Ingeniería en Biotecnología Molecular", and more recently the Molecular Biotechnology PhD Program at University of Chile. He has also directed several graduate programs at University of Chile: Biochemistry (1988-1992 and 1996-2000); Molecular and Cellular Biology (1992-1996); Molecular and Cellular Biology, and Neurosciences (1996-2001) and Microbiology (2007-). He has mentored the thesis of many undergraduate and graduate students that now are doing research in academic and/or applied fields at national and international prestigious institutions. Finally, he has been President of the Biochemistry and Molecular Biology Society (1991-1992); Sociedad de Biología de Chile (2005-2006) and the Latin American Protein Society (LAPS) (2010-2013). This last Society is recognized by the American Protein Society.

Severo Ochoa Lecture

Architecture of large macromolecular complexes in DNA Repair using cryo-electron microscopy (cryo-EM).

Llorca O[1], [1]Structural Biology Programme, Spanish National Cancer Research Centre (CNIO), C/ Melchor Fernández Almagro, 3. 28029 Madrid Tel: (34) 917 328 000 ext 3000 ollorca@cnio.es.

Our group is dedicated to improve the mechanistic understanding of large macromolecular complexes important in DNA repair and DNA damage signalling. Large multi-subunit macromolecular complexes perform many of these processes. Defining the architecture, 3D structure and conformational changes of these large complexes provides relevant information about how they function. Cryo-electron microscopy (cryo-EM) methods can now be used to resolve high-resolution structures of these complexes, but dealing with structural flexibility and heterogeneity is still a major issue in order to reach high resolution. During my talk, I will introduce recent advances in the cryo-EM methodology, and where this field is moving. I will describe examples from our current research in the laboratory to introduce the potential of this methodology, but also major areas of difficulty. I will describe our work in some multi-subunit complexes that combine rigid and flexible regions. Rigid regions are being resolved at high resolution whereas addressing flexibility requires specific image processing strategies.

Oscar Llorca was born on Jan 10th 1968 in Tudela, a medium-size city in Navarre, in the north of Spain. After 1-year as a student at a High School in St. Louis, Missouri (USA) in 1986-1987, he returned to Navarre for his graduate studies in Biology. Llorca graduated in the University of Navarre in 1992, and then moved to Madrid for his PhD studies. In 1996, Oscar Llorca obtained his Ph.D. in Molecular Biology at the National Centre for Biotechnology (CNB) in Madrid under the supervision of JL Carrascosa and JM Valpuesta. At the CNB, Llorca performed influential work on the structural characterization of prokaryotic and eukaryotic chaperonins using electron microscopy. This work was published in Nature, Nature Structural Biology and EMBO J, and it is highly cited. He joined the Chester Beatty Laboratories (Institute of Cancer Research, London) in 2000 as a postdoctoral scientist in the section of Cell and Molecular Biology supported by a prestigious Marie Curie Fellowship. He characterized DNA repair complexes under the supervision of Keith R. Willison and in collaboration with the Imperial College. In June 2002, Oscar Llorca became a Junior Group Leader at the Centre for Biological Research (CIB) in Madrid, belonging to the Spanish National Research Council (CSIC). The CIB is a multi-disciplinary research institute with a strong Structural Biology Department. At the CIB, Llorca leads a fully independent group and he is internationally recognized as a reference in the study of macromolecular complexes using electron microscopy. Oscar Llorca is full Professor from 2009. Llorca has been in the Directing board of the CIB-CSIC. He is chairman of the panel for Spanish National Research grants in Cell and Molecular Biology. He has been LS1 panel member for ERC Starting Grants in three occasions. His main impact has been made in the applicability of single-particle 3D-electron microscopy to macromolecular complexes involved in DNA repair and RNA degradation. Llorca has also worked in Structural immunology of complement regulation in innate immunity. Llorca has published more than 96 articles in top peer-reviewed journals, including Nature, Nature SMB, Nature Comm., Molecular Cell, Genes and Development, EMBO Journal, Cell reports and PNAS USA, and others.

PABMB Lecture

RNA localization and translation in neuronal axons.

Sotelo-Silveira J[1,2], [1]Genomics, Full Professor, Instituto de Investigaciones Biologicas Clemente Estable.[2]Cell and Molecular Biology, Assistant Proffesor, Universidad de la Republica.

Neuron are highly polarized cells with a high proportion of their cytoplasm compartmentalized in dendrites or axons. It has been a central quest in neurobiology to address fully the mechanisms that sustain the local cell biology of the neuron, far away from the neuronal perikaryon. Pioneering studies performed in the pre-molecular biology era by Edstrom (1962), Koenig (1965, 1979), Giuditta (1980) and Alvarez and Benech (1983) provided the first evidence that axons contain active protein synthesis machinery. Several in vitro studies in regenerating or growing axons point us now, that axons localize a relevant number of transcripts and recent studies focus on gathering direct evidence of the functional role of locally synthesized proteins in axons. Still, what is the mRNA diversity localized into a fully differentiated, myelinated and adult axon is an open question. We have centred our effort in developing methods to characterize the localized transcriptome from micro-dissected adult motor and sensory axons. Initial findings indicate that mRNAs localize into mature axoplasm and the variety of mRNA found is smaller than described for inmature, in vitro growing axons. Although its straight forward to propose that the origin of the localized RNA is the neuronal cell body, we are also studying the possibility of a glial origin of axonal mRNA. Data supporting glia to axon RNA cell to cell transfer will be discussed in the context of a glia/axon functional unit.

ANII, CSIC, PEDECIBA, PEW.

José Sotelo, Instituto de Investigaciones Biológicas Clemente Estable, Uruguay

Prof. Sotelo Silveira has made relevant contributions in the field of neurobiology, especially regarding axonal biology. The work carried out in the last two decades, provided evidence supporting mRNA targeting and local protein synthesis in axons (summarized in Sotelo-Silveira & Holt, 2014). Part of his work characterized the molecular composition of new axonal structures, called periaxoplasmic plaques, described for the first time by Prof. Edward Koenig, where axonal ribosomes were localized. In addition, Dr. Sotelo-Silveira, through the PEW fellowships for Latin American scientists, specialized at the National Cancer Institute (NIH, USA) in genomics. On his return to Uruguay he started a new department focused in genomics at the Instituto de Investigaciones Biológicas Clemente Estable (IIBCE). He continues research in axonal biology (RNA transport and local protein synthesis) and has started lines of research in cancer genomics and microbiology in collaboration with colleagues of IIBCE, Faculty of Sciences and abroad. At the IIBCE he also developed a deep sequencing and bioinformatics platform open to services and collaborations that provides support to the institution and others. Dr. Sotelo-Silveira is an active participant in human resources training activities at national and international levels and he has been president of the Biochemistry and Molecular Biology Society of Uruguay and is currently general secretary of the Pan American Association for Biochemistry and Molecular Biology.

Special Issue Sotelo-Silveira & Holt (Eds.) Local Protein Synthesis in Axons (2014). Dev. Neurobiol. Vol. 74, Issue 3: 207–406.

Tito Ureta prize

Dra. Catherine Connelly, Universidad de Chile, Chile.

Dra. Catherine Connelly retired in 2009 from the University of Chile after a career of 54 years in the Faculties of Science and/or Medicine.
She has permanent residency in Chile since 1964. She received the doctorate from Yale University in 1961.
Dra. Connelly has contributed to academia in Chile by serving over 10 years on the Conecyt/FONDECYT review commisions.
Her scientific works include over 75 publications in international journals in areas of:

- protein synthesis, the use of amphibian oocytes as “test tubes”
- pioneer work at Yale University on the reversible phosphorylation of proteins as a regulatory process.

Dra. Connelly completed **two Postdoctoral** Fellowship in the laboratory of Nobel Prize winner Dr. Marshall Nierenberg of the National Institutes of Health USA (1966-7) and a third fellowship of the American Cancer Society, for an 18 month period (1972-74) at the University of California, laboratory of Dr. Gordon Sato.

Closing Lecture

Chromatin, transcriptional elongation and alternative splicing.

Kornblihtt A[1], [1]IFIBYNE-UBA-CONICET Universidad de Buenos Aires.

Alternative splicing plays key roles in determining tissue- and species-specific differentiation patterns, and in hereditary disease and cancer. The emerging evidence places alternative splicing in a central position in the flow of eukaryotic genetic information in that it can respond not only to various signalling pathways that target the splicing machinery but also to the co-transcriptional control by transcription factors and chromatin structure.

Regulation of alternative splicing by transcription elongation can occur via changes in the RNA polymerase II (RNAPII) molecule itself or in chromatin structure. The first mode is illustrated by the effects of DNA damage caused by UV irradiation on alternative splicing. The UV effect is caused by a systemic response to damaged DNA but not to the damage of the DNA template in *cis*. DNA damage causes inhibition of RNAPII elongation due to hyperphosphorylation of its carboxy terminal domain. We will also show how slow RNAPII elongation can either up- or downregulate the inclusion of cassette exons, depending on the nature of the alternative exon.

The second mode involves changes in chromatin structure linked to specific histone modifications. We will discuss how the deployment of either permissive (H3K9Ac) or repressive (H3K9me2 and H3K27me3) marks differentially regulate alternative splicing and how these mechanisms can be used in therapeutic approaches to hereditary disease.

Alberto R. Kornblihtt was born in 1954 in Buenos Aires, Argentina. He graduated as a biologist (1977) from the School of Sciences of the University of Buenos Aires (UBA) and obtained a PhD in Biochemistry (UBA, 1980) at the Campomar Foundation, supervised by Héctor Torres. He did a post-doc (1981-1984) at the Sir William Dunn School of Pathology in Oxford (UK) with Tito Baralle, where he cloned the human fibronectin gene and found its alternative splicing. He is Plenary Professor at the Department of Physiology, Molecular and Cell Biology (DFBMC)[2] of the of the FCEN and Director of the Institute of Physiology, Molecular Biology and Neurosciences of the National Research Council (IFIBYNE-UBA-CONICET) of Argentina. Since 2002 he is an International Research Scholar of the Howard Hughes Medical Institute (HHMI). He is a Foreign Associate of the National Academy of Sciences of the USA and a member of EMBO. He served the Board of Reviewing Editors of Science (2010-2015). He is also a member of the Argentine National Academies of Sciences and of Exact and Natural Sciences. He was awarded the Guggenheim fellowship (1991), the Konex Platinum Award (2003 and 2013), a chair from the Fundación Antorchas (2000-2008), the Bicentennial Medal (2010), the Houssay Achieving Award in Chemistry, Biochemistry and Molecular Biology (2010), the prize Investigator of the Argentine Nation (2010), granted by the President of Argentina, the Honorary Mention Domingo Faustino Sarmiento of the Argentine Senate (2011), the TWAS prize in Medical Sciences (2012) and the Diamond Konex award as the most relevant scientist of the decade of his country (2013), ex aequo with the theoretical physicist Juan Martín Maldacena. He served the National Committee on Ethics in Science and Technology of Argentina (CECTE). He supervised 17 PhD theses, organized 5 international scientific meetings, including co-charing the Cold Spring Harbor Laboratory meeting on RNA processing in 2017, 2019 and 2021. He is Editor-in-Chief of the journal Transcription and acted as President of the Argentine Society for Biochemistry and Molecular Biology (SAIB) for the term 2010-2011.

Symposium

Gene expression and Molecular Biosystems

Chair: Luis Larrondo

Mapping a genetic wiring diagram of a cell.

Andrews B[1], [1]The Donnelly Centre for Cellular and Biomolecular Research, Faculty of Medicine, University of Toronto. Toronto, Canada**.**

Genome sequencing projects are providing an unprecedented view of human genetic variation. There is growing awareness that genetic interactions, involving combinations of polymorphic alleles, may play a major role in determining phenotype. Yet, our understanding of the principles of the genetic networks relevant to human disease remains rudimentary. To address this challenge, our group has focused on identifying genetic interactions in a model eukaryote, the budding yeast, Saccharomyces cerevisiae, which provides a unique format for exploring genetic networks. Our group developed the 'synthetic genetic array' (SGA) method, which automates yeast genetics and enables systematic mapping of genetic interactions. We have used SGA to complete a reference genetic interaction map for yeast. In total, we examined ~23 million pairs of deletion mutants and mutant strains carrying temperature-sensitive (TS) alleles of essential genes, mapping nearly 1 million genetic interactions. This network provides an unprecedented view of the functional organization of a cell and elucidates fundamental properties of genetic networks, which we leveraged to develop innovative approaches to discover network hubs and pleiotropic genes. Insights gained from the yeast network have inspired methods for interpreting human genotyping data and discovering combinations of common genetic variants that reflect disease risk.

Brenda Andrews is the Charles H. Best Chair of Medical Research, Director of the Donnelly Centre for Cellular and Biomolecular Research and Professor of Molecular Genetics at the University of Toronto. Dr. Andrews completed her PhD in Medical Biophysics (with Paul Sadowski) at the University of Toronto, and postdoctoral training in genetics with the late Dr. Ira Herskowitz at the University of California San Francisco. In 1991, Dr. Andrews was recruited to the Department of Medical Genetics (now Molecular Genetics) at the University of Toronto. She became Chair of the Department in 1999, a position she held for 5 years before assuming a position as Chair of the Banting & Best Department of Medical Research and as the inaugural Director of the Donnelly Centre. Dr. Andrews' current research interests include analysis of genetic interaction networks in budding yeast and mammalian cells, using high through-put genetics platforms that include high content microscopy for systematic analysis of cell biological phenotypes. Dr. Andrews is a Companion of the Order of Canada, an elected Fellow of the Royal Society of Canada, the American Association for the Advancement of Science and the American Academy of Microbiology.

Sequence specificity of unconventional RNA binding proteins.

Hughes T[1], [1]Donnelly Centre, Medicine, University of Toronto.

RNA-binding proteins (RBPs) participate in diverse cellular processes and have important roles in human development and disease. The human genome encodes hundreds of unconventional RNA binding proteins (ucRBPs), which physically associate with RNA but lack common RNA-binding domains (RBDs) such as RRM, KH, Pumilio, and CCCH zinc fingers. The degree to which these proteins bind RNA in a sequence specific manner is unknown. We previously described RNAcompete, a method for identifying the sequence preferences for RBPs, and applied it to generate a compendium of over 200 RNA binding motifs for eukaryotic RBPs that contain canonical RBDs. We are now applying RNAcompete to analyze human ucRBPs. A random sampling of 220 identified 26 with apparent sequence preferences (i.e. motifs). Thus, a subset of ucRBPs possesses intrinsic RNA sequence specificity. All 26 have previous literature supporting association with RNA, or contain nucleic acid binding domains, however. The motifs predict binding data from living cells, where available. Ongoing efforts are aimed at analysis of an expanded panel of ~500 ucRBPs, identification of RNA binding regions, and additional comparisons of in vitro and in vivo RNA binding.

NIH (USA) CIHR (Canada)

Timothy R. Hughes is a Professor in at the Donnelly Centre for Cellular and Biomolecular Research at the University of Toronto. He studied engineering and music at the University of Iowa, and received his Ph.D. in Cell and Molecular Biology from Baylor College of Medicine, working on telomere replication. He did his postdoctoral work at Rosetta Inpharmatics (now Merck) working on microarray technology and its applications, including the development of ink-jet arrays now available from Agilent. Since moving to Toronto in 2001, Dr. Hughes has been the recipient of a Canada Research Chair in Genome Biology, the Ontario Premier's Research Excellence Award, the Terry Fox Young Investigator award, and an HHMI foreign scholarship. He has authored or co-authored over 150 manuscripts, and is a scholar of the Canadian Institutes For Advanced Research. His laboratory works in gene regulation, systems biology, RNA processing, genome sequencing, and computational biology, and has a fundamental goal of understanding how the genome sequence is interpreted by the cell.

Imagine the future: from aging yeast cells to multifunctional proteins.

De Luna A[1], [1]Unidad de Genómica Avanzada (Langebio) CINVESTAV Mexico.

Aging is a complex trait with clear genetic underpinnings. With a growing number of genetic aging factors in hand, the next great challenge is to describe how such cellular processes and regulatory pathways are integrated with one another and how they are affected by environmental cues. In our lab, we have developed automated tools based on robotic integration to systematically identify aging factors in the budding yeast *Saccharomyces cerevisiae* and to score their genetic interactions (epistasis). In this talk, I will present new insights into the gene-network wiring of aging cells, including a role of the Swr1 histone-exchange complex in post-mitotic cell survivorship and a functional link between autophagy and the Arv1-mediated lipid-homeostasis pathway. Within this context, I will present an intriguing case of protein multifunctionality revealed by non-catalytic phenotypes of metabolic enzymes. Finally, I will describe a genome-wide screen for genetic mediators of longevity by dietary restriction, underscoring the relevance of cell-cycle control as a mechanism for chronological longevity. This study provides a global view of the cellular response to a non-pharmacological intervention that extends the lifespan of organisms, from yeast to humans.

Alexander De Luna is a Research Professor at CINVESTAV in Irapuato, Mexico. He got his PhD in Biomedical Science at the Universidad Nacional Autónoma de México and moved to the Department of Systems Biology at Harvard Medical School to work as a Pew postdoctoral fellow with Roy Kishony. His research has focused on the fields of genetics, evolution, metabolism, and aging. Currently, his lab aims to contribute in understanding how genes, the environment, and their interactions influence complex biological traits, with special interest on cellular aging. In 2012 he was named Young Affiliated Member of the TWAS Academy of Sciences and since 2016 he is member of the ACAL Latin American Academy of Sciences.

Gene expression and circuitry in synthetic and natural network designs

Goity A[1], Olivares-Yañez C[2], **Larrondo L[2]**, [1]Genetica Molecular y Microbiologia, Ciencias Biologicas, Pontificia Universidad Católica de Chile.[2]Genetica Molecular y Microbiologia, Ciencias Biologicas, Pontificia Universidad Católica de Chile.

In the fungus *Neurospora crassa*, as in other model organisms, synthetic biology based-strategies have been seldom adopted for the study of circadian oscillators. Our current efforts on this matter have focused on examining the genetic plasticity of the Neurospora circadian clock through transcriptional rewiring. This design implies the addition of new positive elements (transcription factors) that are now integral part of a hybrid oscillator (HO) that mixes canonical and new components. Remarkably, this HO free-runs, has a period close to 24 h, is temperature compensated and it is entrainable by external cues. Such an approach is already revealing important insights regarding time-delay mechanisms and alternative design principles compatible with clock function. On the other hand, we have adopted optogenetic approaches to further delve into Neurospora's circadian and light-responses. In doing so, we had the ability to turn this fungus into a "*live canvas*" on top of which images can be projected causing a bioluminescent biological response that recreates the original image with great precision. Remarkably, since this optogenetic circuit is integrated in the Neurospora circadian regulatory network, the fungus reproduces on subsequent days -in a circadian manner- the image that it had originally "seen", creating an eidetic (photographic) memory effect. Such phenomenon, based on local discrete phase changes, not only will provide new insights on phase responses, but it also allows for the opportunity to ponder on concepts such as vision and memory.

FONDECYT 1171151 and MN-FISB NC120043.

Luis Larrondo received a Ph.D in Cellular and Molecular Biology at the P. Universidad Católica de Chile (PUC). 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 went back Chile, where he is now and associate professor and the director of the Millennium Nucleus for Fungal Integrative and Synthetic Biology (PUC). His lab works with different fungal systems studying the molecular mechanisms underlying biological oscillators, assessing the impact that circadian clocks and light-regulation 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. The lab is also interested in the effect of combining different transcriptional modules in order to obtain tunable and predictable behaviors upon external stimuli.

Symposium

Molecular host-pathogen interactions
Chair: Alexis Kalergis

Novel Strategies for Enhancement of Human TB Immunity.

Hoft D[1], [1]Infectious Diseases, Allergy & Immunology St. Louis University.

Bacillus Calmette-Guérin (BCG) vaccination provides only limited protection against tuberculosis (TB), and boosting TB-specific CD4+ Th1 cells with new vaccines has not improved protection. Therefore, novel targets and approaches for TB vaccination are needed. Our group is exploring the potential for mucosal immunity and unconventional T cells to improve vaccine immunity.

We compared the oral (PO) and/or intradermal (ID) BCG vaccination in healthy human subjects. Th1 responses capable of IFN-g production were more strongly induced by ID BCG. In contrast, mucosal responses (TB-specific secretory IgA and alveolar T cells) were induced only by PO BCG vaccination. Early gene signatures in BCG-induced CD4+ memory T cells were largely distinct comparing PO and ID vaccinated groups. Ongoing systems biology analyses are investigating correlations between specific gene expression patterns, and the mucosal vs systemic immune responses induced, which will greatly inform future TB vaccine development.

$\gamma_9\delta_2$ T cells provide a natural bridge between innate and adaptive immunity and may serve as potent targets for TB vaccines and/or immunotherapy. We established a novel strategy to identify TB antigens responsible for expansion of protective $\gamma_9\delta_2$ T cells. Organic extractions of *Mycobacterium tuberculosis* lysates purified polar lipid fractions with enriched biologic activity. A combination of Mass Spectrometry and Nuclear Magnetic Resonance analysis of bioactive fractions identified 6-*O*-methyl-glucose containing lipopolysaccharides (mGLP) as key components in bioactive fractions. We now are investigating whether mGLP can induce protective $\gamma_9\delta_2$ T cells in nonhuman primates. These results have important implications for development of new immunotherapeutic approaches for prevention/treatment of TB.

Funding: PleaselistourVTEUcontract, mygamma/deltaTcellRO1, mygamma/deltaTcellGatesawardandtheSilver/HoftRO1.

Dr. Daniel F. Hoft is a Professor and Director of the division of Infectious Diseases, Allergy & immunology in the department of Internal Medicine of Saint Louis university School of Medicine. He Started His Career in Science graduating in 1977 with a bachelor of Chemistry at Grinnell College, Iowa, He attended the university of Missouri, where he graduated in Medicine in 1985. He returned to Iowa to pursue a Ph.D. at the University of Iowa, where he graduated in the Immunology and Microbiology program in 1992, after many years dedicated to the University of Iowa Dr. Hoft was granted the Dianna and J. Joseph Adorjan Endowed Chair of Infectious diseases and Immunology. Dr. Hoft has led 14 NIH grants, as well 15 human vaccine trials with Bovine Calmette-Guerin and other types of vaccines. Among Dr. Hoft Work he has Developed Vaccines for Chagas Disease, Improved Tuberculosis vaccines and Pandemic Influenza Vaccine development. Dr. Horf current NIH-funded work focuses on investigations of the basic mechanisms of mucosal and systemic immunity protective against mucosally transmitted, chronic intracellular pathogens. Most of his research involves 3 human pathogens: *Mycobacterium tuberculosis* (Mtb), the protozoan parasite *Trypanosoma cruzi* and influenza. Dr. Hoft has published more than 80 publications in different highly respected Journals, including, Journal of Immunology, Cancer Research, Human vaccines & immunotherapeutics, Vaccine and PLoS Pathogens, among others.

Establishing Non-specific T cell Responses to Bacterial Infection using Pet Shop Mice.

Labuda J[1], Pham O[1], Tsolis R[1], **McSorley S[1]**, [1]Center for Comparative Medicine, Department of Anatomy, Physiology and Cell Biology, School of Veterinary Medicine, University of California Davis, Davis, CA 95616, USA.

Immunity to intracellular bacteria infection requires the activity of protective CD4 T cells but the relevance of cognate versus non-cognate activation of these cells is unclear. We have used a variety of mouse models to examine the activation of CD4 Th1 cells using TCR-independent stimuli. A co-housing model that allows transmission of irrelevant infectious agents to in-bred SPF mice provides a simple normalization process to induce memory cell formation and influence subsequent resistance to infection. Furthermore, our data show that CD4 Th1 cell expression of both IL-18R and DR3 is required for optimal IFN-γ induction, suggesting relatively non-specific signals amplify the activity of bacterial-specific T cells. Mice lacking T cell intrinsic expression of MyD88, have higher bacterial burdens upon infected with either Salmonella, Chlamydia or Brucella, suggesting that non-cognate Th1 stimulation is a critical element of efficient bacterial clearance. Together, these data point to the unappreciated importance of non-specific stimuli in the establishment of immunity to intracellular bacteria.

Dr. Stephen Mcsorley Is a professor in the Center of Comparative Medicine of the University of California Davis, where he has been an interim director during 2015-2016. He started his career in Scotland in 1992 when he graduated in Immunology at the University Of Glasgow, Place where he pursued his PhD. of Immunology and Microbiology. Dr. Mcsorley traveled to France for his first postdoctoral fellow at the ***Institut de Pharmacologie Moléculaire et Cellulaire*** from the National Center for Scientific Research of the French ministry of Education and Research, after completing his work in France, Dr. Mcsorley was granted another Postdoctoral fellow at University of Minnesota, where he became associate professor for 6 years. He has over 20 years of experience examining antibody and CD4 T cell responses to bacterial infection, primarily using mouse models, but also more recently with clinical samples. Since accepting my first faculty position in 2002 at the University of Connecticut Health Center. Dr. Mcsorley has contributed to the development of antigen-specific tools for bacterial immunity, the analysis of the innate immune response to flagellin and analysis of Salmonella-specific B cells; becoming Fellow of the American Academy of Microbiology. Dr. Mcsorley has published over 19 peer-reviewed research papers and 12 reviews over the last 5 years.

Preventing RSV Vaccine-Enhanced Immunopathology.

Varga S[1], [1]Department of Microbiology The University of Iowa.

Respiratory syncytial virus (RSV) is the leading cause of severe respiratory tract infection in infants and young children. CD8 T cells play a critical role in mediating viral clearance following an acute RSV infection. While memory CD8 T cells provide protection from re-infection from respiratory viruses such as influenza and SARS, the relative contribution of memory CD8 T cells in providing protection against RSV remains unclear. To generate high magnitude CD8 T cell memory in the absence of CD4 T cell memory and antibodies, we immunized naïve mice with dendritic cells pulsed with an RSV-derived peptide followed by a boost with a recombinant *Listeria monocytogenes* expressing the same RSV-derived epitope. Memory CD8 T cells significantly reduced viral titers following RSV challenge, but did so at the expense of increased airway dysfunction, weight loss, and mortality compared to controls. Importantly, the severe immunopathology and mortality observed was specific to the context of an RSV infection, as prime-boosted mice challenged with a recombinant influenza virus expressing the same RSV-derived epitope did not exhibit enhanced disease. The induction of a pro-inflammatory cytokine storm mediated by TNF and IFN-γ was observed in the serum of prime-boosted mice following RSV challenge. Additionally, RSV-specific memory CD8 T cells produced large amounts of IFN-γ locally within the lung, and adoptive transfer of wild-type but not IFN-γ-deficient memory CD8 T cells resulted in enhanced airway dysfunction and weight loss. Our results indicate that memory CD8 T cells are able to mediate protection against RSV infection. However, memory CD8 T cells acting alone in the absence of antibodies and memory CD4 T cells induce significant immunopathology and mortality through the induction of a systemic pro-inflammatory cytokine storm and local IFN-γ production.

Dr. Steven M. Varga is a Professor of the department of microbiology and director of the interdisciplinary graduate program in Immunology at the University of Iowa, He started his career in sciences at the University of Notre Dame, where he graduated in 1993 as a Bachelor in Biology, later he followed his career with a Ph.D. program of Immunology and Virology at the University of Massachusetts, graduating in 1999, he followed with a Postdoctoral Fellow, at the Carter Immunology center from the University of Virginia under the supervision of J. Braciale, where he was granted the National Research Service Award from NIH in 2000-2002. Dr. Varga's laboratory studies the contribution of virus-specific T lymphocytes to enhanced disease and immunopathology during virus infection. A major focus of the laboratory is gaining a better understanding of the immune response to respiratory syncytial virus (RSV) infection. RSV is the most common cause of bronchiolitis and pneumonia in young children worldwide. Attempts to create an RSV vaccine have been unsuccessful to date. Administration of a formalin-inactivated vaccine to children results in exacerbated morbidity and mortality upon natural RSV infection. It is believed that the immune system is responsible for the enhanced disease that was exhibited in the vaccinated children. The laboratory is currently studying the mechanisms by which virus-specific CD4 T cells mediate damage within the infected lung. By better understanding how T cells cause damage to the host during infections we hope to be able to devise novel vaccination strategies that will limit the destruction of host tissue during an immune response against a virus or parasite. Dr. Varga has published more than 62 articles in distinguished journals, including, Journal of Immunology, Journal of Virology, Immunity, Cell Immunity, American Journal of Respiratory Cell and Molecular Biology, Future Virology, Virology, PLoS pathogens, Pediatric Research.

Interference with immunological and neurological synapses as virulence mechanisms of RSV. Implications for vaccine design.

Kalergis A[1], [1]Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

Human Respiratory Syncytial Virus (hRSV) is the leading cause of pneumonia in young children worldwide. hRSV can cause severe symptoms both in the respiratory and nervous systems, such as bronchiolitis and encephalopathy, respectively. We have observed that hRSV infection prevents T cell activation by antigen-presenting cells, by a mechanism involving the nucleoprotein. This protein is expressed on the surface of epithelial and dendritic cells, reduces TCR signaling and pMHC clustering and prevents immunological synapse assembly by T cells. These data suggest that hRSV nucleoprotein expression at the cell surface of infected cells alters T cell activation, which could work as a major virulence factor by impairing host immunity and enhancing susceptibility to reinfection by hRSV. Based on these findings a hRSV vaccine was generated, which induces protective immunity in various animal models and is currently undergoing evaluation in human clinical trials. Furthermore, hRSV infection causes a learning impairment due to inflammation at the central nervous system (CNS) and reduction of long term potentiation in the hypocampus of infected animals. Due to alterations on the blood brain barrier after hRSV infection, elements of the immune system enter the CNS impairing the normal function of neurons and astrocytes in mice. These data from laboratory animals have prompted studies to evaluate whether hRSV infection can affect learning in humans.

FONDECYT 1070352 and the Millennium Institute on Immunology and Immunotherapy

Dr. Alexis M. Kalergis is a Full Professor at the Department of Molecular Genetics and Microbiology, School of Biological Sciences and the Department of Endocrinology, School of Medicine, at the Pontificia Universidad Católica de Chile. Dr. Kalergis is a Biochemistry graduate from P. Universidad Católica of Chile and obtained his MSc and Ph.D. in Microbiology and Immunology from the Albert Einstein College of Medicine in New York, where his graduate work was awarded the Julius Marmur Award. Then, he performed as a post-doctoral trainee at the Albert Einstein and The Rockefeller University, supported by an Irvington and a Helen Hay Whitney Fellowships. Dr. Kalergis has been Director of the Millennium Nucleus and Institute on Immunology and Immunotherapy, which was recently appointed as a Center of Excellence by the Federation of Clinical of Immunology Societies and Dr. Kalergis as a member of the Board of Directors of this organization. He was appointed as a member of the Presidential Science Committee and the Senate Committee on Science. Further, he was recently nominated as Scientific Liason ICGEB and Director of the Biotechnology Committee at CONICYT. Dr. Kalergis and his colleagues work on the technological transfer of basic biomedical knowledge to concrete applications consisting of new therapy tools for diseases that are prevalent in the Chilean population. As an example, the group of Dr. Kalergis has recently developed a new vaccine against Respiratory Syncytial Virus, the microbe responsible for over 80 % of hospitalization due to respiratory failure. This vaccine (made in Chile) is about to enter a phase I clinical trial in collaboration with physicians and international foundations that contribute to global human health. The research of Dr. Kalergis focuses on the molecular interactions that regulate the synapse between T cells and dendritic cells and their role in immunity against pathogens and tumors, as well as on their modulation to restore self-tolerance during autoimmune disorders. Dr. Kalergis has published over 160 articles in leading journals and over 55 patent applications.

Symposium

Cono sur symposia: Plants and their environment
Chairs: Claudia Stange-Patricio Ramos-Luis Morales

Morpho-physiological and biochemical responses of *Colobanthus quitensis* populations to salinity (NaCl) and copper ions (II) under controlled culture conditions.

Cuba-Díaz M[1], Klagges M[1], Castel K[1], Marín C[1], Arriagada P[1], Machuca Á[1], Cabrera G[2], [1]Ciencias y Tecnología Vegetal, Escuela de Ciencias y Tecnologías, Universidad de Concepción.[2]Grupo Hijuelas Centro de Investigación, Desarrollo e Innovación .

Colobanthus quitensis is the only native dicotyledonous in Antarctica, but also is distributed latitudinal and altitudinal from Antarctica to the south of Mexico. Abiotic factors such as constant sea spray, high tides, high metal ion content and acidic pH are presented in several of the species habitats. It could be assumed that responses to these environmental factors can be differential according to the habitat. The effect of NaCl and Copper ions (II) was assessed on *C. quitensis* populations through morpho-physiological and biochemical variables *in vitro* and in common garden. In response to NaCl, only two populations showed morphological and physiological symptoms of stress *in vitro*, whereas in the common garden only the Antarctic population showed greater sensitivity in all variables evaluated. In the four populations the content of photosynthetic pigments decreased and the content of proline, total soluble sugars and sucrose increased. In the presence of Cu (II), the populations of Punta Arenas and Antarctic were the least affected while the Andean populations showed a significant decrease in their morphology. Proline content increased significantly in Andean populations, whereas CAT and POD activity and MDA concentration increased and the content of photosynthetic pigments decreased in the four populations. There were no changes in antioxidant capacity and total phenolic compounds in any of the populations studied in the presence of NaCl and Cu^{+2} but all showed a considerable increase of catechins. We can conclude that populations showed different responses to both abiotic agents in relation to the conditions of their habitat.

(Sponsored by Proyectos VRID 213.418.004-1.0, INACH RT_02-09)

Marely Cuba Díaz graduated with a degree in Biology at the Universidad de La Habana (Cuba) in 1993. Afterwards she worked in somatic embryogenesis of coffee and the development of tissue culture and bioreactor culture protocol in crops and ornamental species at the Instituto Nacional de Ciencias Agrícolas (Cuba). After a short stay at CINVESTAV Irapuato (Mexico), under the direction of Luis Herrera Estrella, she was awarded a Master degree in Plant Biology and Biotechnology at the Universidad de La Habana (Cuba), which was based on the implementation of new protocols of genetic transformation of plants through biobalistics. Then she completed a PhD at the Universidad de Chile in 2004 where she studied the regulation of genes overexpressed in response to cold in *Deschampsia antarctica*, the only native grass that lives in Antarctica. After that, she worked for a short period advising private projects in the floriculture area, supporting the development of specialized protocols in some Conicyt projects and teaching at several universities. Since 2007, she has joined the Los Angeles Campus of the Universidad de Concepción where she has led and participated in several research projects. She is currently in charge of the Laboratory of Biotechnology and Environmental Studies of the same Campus. She returned to the field of Antarctic plants and is interested in studying the mechanisms that allow the Antarctic plants withstand the harsh environmental conditions in the Antarctic ecosystem and in other habitats where these species live and, moreover the evaluation of the physiological, ecological and molecular aspects that allow the resilience of these plants to its current ecosystem conditions and facing the changes due to climate regional change. In addition, she obtained a valuable germplasm collection of Antarctic plants and others Chilean populations of same species which, not only using them for her scientific research and collaborations, but she uses them to develop protocols directed to the potential biotechnological uses from these plants.

UV-B effects in plant development.

Casati P[1], [1]CEFOBI Universidad Nacional de Rosario-CONICET.

Plants are exposed to a broad spectrum of environmental stimuli throughout their life time, being sunlight the main source of energy and information. UV-B is a component of the solar radiation that induces a number of modifications in plant growth and development. UV-B radiation inhibits *A. thaliana* (a dicot plant) growth by inhibiting cell division in proliferating leaves. UV-B also affects maize (a monocot) growth. Our results demonstrate that the decrease in leaf growth in UV-B irradiated leaves is a consequence of a reduction in cell production, and a shortened growth zone (GZ). To determine the molecular pathways involved in UV-B inhibition of leaf growth, we performed RNA sequencing on isolated GZ tissues of control and UV-B exposed plants. Our results show a link between the observed leaf growth inhibition and the expression of specific cell cycle and developmental genes, including *Growth Regulating Factors* (*GRFs*) and transcripts for proteins participating in different hormone pathways. On the other hand, UV-B also induces changes in flowering time. We demonstrated that Arabidopsis plants grown under white light supplemented with UV-B show a delay in flowering time. Using a combination of gene expression analysis and UV-B irradiation of different flowering mutants, we gained insight into the pathways involved in the observed flowering time delay in UV-B exposed Arabidopsis plants. Modification in the expression of several flowering time genes was determined. UV-B exposure of flowering mutants supports the involvement of the PRC2 complex in the observed delay in flowering time, mostly through the age pathway.

Paula Casati. Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)-Universidad Nacional de Rosario (UNR), Rosario, Argentina. Paula Casati was born in 1971 in Rosario (Argentina), and also grew up and now lives in Rosario. She studied Biotechnology in the Faculty of Biochemistry (UNR), from 1990-1994, where she did her undergraduate Thesis at the CEFOBI, investigating the metabolism of C4 compounds in plants. Then, she did her PhD Thesis at the same Institute from 1995-1998, where she continued her research on C4 metabolism. She also then started investigating the effect of UV-B radiation in plants during 2 short visits to Dr. Gerald Edwards lab at the Washington State University, USA. In 2001, and after a short postdoctorate at the CEFOBI, she joined the lab of Dr. Virginia Walbot at the Department of Biology, Stanford University, as a postdoctoral student from 2001-2005. During her postdoctoral training, she characterized different pathways affected after UV-B exposure in maize, including changes in secondary metabolism, in the translation machinery and in the chromatin. By use of different maize inbreds and landraces, as well as transgenic plants with altered expression of genes participating in diverse UV-B responses, she could identify several new participants in the plants responses to this radiation. In 2005, Paula came back to Argentina, where she started her lab investigating different aspects of UV-B responses in plants species, including maize and Arabidopsis. The different areas of her current research are flavonoid metabolism and regulation in maize, chromatin remodeling after UV-B exposure, cell cycle regulation and development regulation by UV-B, and the investigation of ribosomal proteins roles after exposure to this radiation.

Cell membrane function and drought tolerance in plants.

Borsani O[1], [1]Biología Vegetal, Facultad de Agronomía, Universidad de la República.

Plants as sessile organisms should be adapted for a wide range of changes in the surrounding environment, so they have several mechanism focused to minimize the impact of this changes. The limited success in classical breeding programs to increase tolerance to environmental stress factors has spurred efforts to understand the physiological mechanisms and the genetic regulation abiotic stress. In this context we focus our work in the elucidation of new components involved in plant abiotic stress tolerance. We previously uncovered a role for squalene epoxidase 1 (SQE1) in root sterol biosynthesis and drought tolerance. Build on our prior study we determine at the cellular level how membrane integrity and composition could affect cellular function. The study in the root hair (a unique cell) of the Arabidopsis dry2/sqe1 mutant (with less sitosterol and stigmasterol) showed how sterol composition affects membrane order “in vivo” using fluorescent probes in combination with microscopy techniques. Moreover, trying to assess if membrane function is conserved in the mutant we investigated the location and activity of proteins that lead root hair polar growth and vesicular trafficking. We found that changes in membrane fluidity, generated by changes in sterols profiles, affect the location and function of membrane proteins, endocytosis and vesicular traffic process. Analysis of the impact of membrane order in plant cell environmental responses open new possibilities to investigate the role of membrane in the stress perception mechanisms by plants.

(Sponsored by CSIC-Grupos I+D UdelaR)

Omar Borsani obtained the undergraduate studies in Agricultural Engineering at the Universidad de la Republica (Uruguay) in 1993 and received the title of Master of Science in 1998 (PEDECIBA). He got the degree of Doctor of Science by the University of Málaga (Spain) in 2002 in a topic related with salt and osmotic stress tolerance mechanisms in models and cultivated plants. After that he has profited postdoctoral stays at Dept. Botany of the University of California-Riverside and Dept. Biochemistry and Molecular Biology of University of Malaga (2002-2006). He is currently Full Professor of Biochemistry and Plant Physiology at the Department of Plant Biology of the Faculty of Agronomy (Uruguay). He leads of “Abiotic Stress in Plants” Research Group and coordinates the National Agricultural Biotechnology Network. His work has contributed to understand new mechanisms involved in the abiotic stress tolerance in plants with focus in the transference of this knowledge to plant phenotyping applied in the breeding programs.

Across the ocean from Taiwan to Easter Island: tracking genetic diversity of extant and historic paper mulberry (*Broussonetia papyrifera*) to understand its dispersal history.

Seelenfreund D[1], Peñailillo J[1], Payacan C[1], Olivares G[1], Moncada X[2], Seelenfreund A[3], [1]Departamento de Bioquímica y Biología Molecular, Ciencias Químicas y Farmacéuticas, Universidad de Chile.[2]Centro de Estudios Avanzados en Zonas Áridas CEAZA.[3]Departamento de Antroplogia Universidad Academia de Humanismo Cristiano.

Paper mulberry (*Broussonetia papyrifera*), a dioecious tree native to East Asia was introduced into the Pacific in prehistoric times by human translocation, as far east as Easter Island. Its bark is used as a source of fiber for making bark-cloth textiles of practical and symbolic importance. Therefore, paper mulberry may have been the most widely transported fiber crop in prehistory.Because paper mulberry is propagated asexually in the Pacific, its dispersal between islands was totally dependent on humans. We analysed the genetic diversity of contemporary plants, historical herbaria and bark-cloth samples using several genetic markers to understand the human-mediated dispersal of this species. Our results show that Pacific paper mulberry has a clear Taiwanese origin and that extant plants are mainly female, however in the past also male plants were present. Despite a short timescale, a common Asian origin and asexual propagation, we have found limited genetic diversity within Remote Oceania. Furthermore, subtle signatures within the paper mulberry genome allow discerning separate regions within the Pacific, such as East and West Remote Oceania.Interestingly, an overview of the genetic diversity of contemporary and historical specimens of paper mulberry in Remote Oceania shows significant erosion of genetic diversity in extant plants.As paper mulberry plants accompanied people across the full range of human expansion, the study of the genetic diversity of paper mulberry in the Pacific also serves as a proxy for human migration.

Supported by grants FONDECYT 1080063 and 1120175 to AS; Visiting Scholars Program, Taiwan and PEEI/UChile to DS.

Dra. Daniela Seelenfreund is a graduate in Biochemistry from the Universidad de Chile. She obtained her PhD in 1989 at the Pontificia Universidad Católica de Chile under the supervision of Dr. Rafael Vicuña, studying the contribution of Streptomyces on lignin biodegradation. Since 1989 she is employed at the Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile. From 1990 to 1997 she worked with Luz María Pérez' group on the response of Citrus limon against fungal pathogens. She also continued collaborating with Rafael Vicuña's group working on lignin biodegradation from 1997 until 2002. From 2004 to 2010 she worked with Pilar Durruty (San Juan de Dios Hospital) on the genetics of diabetes complications, particularly on polymorphisms associated to diabetic nephropathy. Currently, she is involved in a new research line that uses genetic tools to understand past human history. In particular, the settlement of the Pacific by tracking the Austronesian expansion using paper mulberry (Broussonetia papyrifera) as a proxy of human movement. This plant, native to Asia, was and is still used as a valuable source of textiles. To identify the geographic origins of paper mulberry and reconstruct its human-mediated spread through Island Southeast Asia and into the Pacific, the genetic diversity of contemporary paper mulberry, herbarium specimens and historic textiles have been analysed using diverse genetic markers. These analyses have uncovered dispersal patterns of paper mulberry in the Pacific that are in agreement with archaeological evidence of human migration and interactions. She currently coordinates the Master's program in Biochemistry and holds the position of Head of the Department of Biochemistry and Molecular Biology at the Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.

Dra. 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. Patricio Ramos studied Biochemistry at the University of Concepción and obtained his professional degree in 2003. He obtained his PhD in engineering in plant genetics from the University of Talca in 2010 and then he performed a post-doctoral training at the Institute of Biological Sciences (ICB) at University of Talca. In 2011 started an insertion in the academy program (PIA) and now he works at University of Talca in “The scientific multidisciplinary nucleus” and is part of the academic team of the ICB and PhD program. One of the main research lines is focusing in the study of phenylpropanoid metabolic pathway regulation and the relationship with hormonal signaling. Phenolic compounds, specifically flavonoids, display wide biological effects such as modulation of the interaction between plants and microorganisms, stress response, pigmentation as well as the hormonal transport regulation in several plant tissues. Homeostasis of those compounds within the subcellular compartment and balance between lignin and flavonoid is part of his scientific research interest.

On the other hand, phenylpropanoid displays several health’s benefit to consumers, the study of biosynthesis and accumulation in fruits and other edible plant tissues are also part of his research line.

Dr. Luis Morales Quintana studied Engineering in Bioinformatic at the University of Talca and received his degree in 2009. He performed a PhD in Science with mention in plant genetic engineering from the University of Talca and received the degree at 2013. In 2014, he obtained a postdoctoral position in University of Talca through a program of insertion in the academy of CONICYT (PAI/Academia), where now work as professor at the Institute of Biological Sciences.

His research main topic focuses in the study of molecular aspects in the formation and degradation of plant cell wall. The strategies used involve: structural bioinformatics, biochemical characterization, kinetics studies, and functional genomics. To describe genes, proteins, enzymes and how they modulate the response to different events occurring in the plant cell wall. It has also been approached the study of enzymes involved in the study of quality traits of fruits, specifically in biosynthesis of volatile compounds related with aroma in strawberry and mountain papaya fruits.

Symposium

Exploring the winding path of cancer

Chairs: Claudia Quezada-Verónica Burzio

Use of liquid biopsy for the detection of molecular alterations in patients with Gliomas.

Ayuso-Sacido A[1], [1]Oncología, Medicina, Universidad San Pablo CEU-Fundación de Investigación HM Hospitales.

High-grade gliomas are the most common primary malignant brain tumours in adults. Despite advances in treatment, the median patient survival rate is 12 to 15 months, as tumour eventually recurs in all patients. Gliomas are commonly detected through clinical assessment and imaging techniques. Tissue specimens are suitable for the evaluation of tumour histopathology at the very beginning of the disease, but they do not allow molecular evolution assessment of the tumour along the course of the disease, which is critical for improving patient survival. Extracellular vesicles (EVs) released from tumours are present in readily accessible biofluids and carry nucleic acids. Tumoral mutated sequences have been recently detected in circulating DNA (ctDNA) and EVs extracted from cerebrospinal fluid (CSF). However, CSF extraction through lumbar puncture is an invasive procedure and not recommended in patients with high intracraneal pressure. Therefore, the identification of tumour-derived nucleic acids within EVs from a more accessible biofluid, like peripheral blood, will provide a useful tool for the diagnostic, follow-up treatment and molecular target identification after tumour progression. In this regard, by using an orthotopic xenotransplant model of human cancer stem cells (hCSCs), we will see how tumour-derived EVs are able to cross the BBB, reach the bloodstream and be successfully used to detect the presence of specific glioma-related mutations.

Dr. Angel Ayuso-Sacido made his PhD at Merck Sharp and Dhome (MSD) and worked as a postdoctoral fellow in the Medicine Department at Mount Sinai School of Medicine, and the Neurosurgery Department at Cornell Medical Center. He come back to Spain and worked as Senior Researcher at Centro de Investigación Príncipe Felipe (CIPF) de Valencia, and Founded the Glioblastoma Spanish Network (REIG). Afterwards, he was Visiting Scientist at Helsinki University and currently, he is the Scientific Coordinator at Fundación de Investigación HM hospitales (FiHM), Director of the Brain Tumour Laboratory at FiHM, Assistant Profesor of Medicine at San Pablo-CEU University, Coordinator of the Nano-Oncology Laboratory at IMDEA nanoscience and President of the REIG.

An alternative model of tumor irrigation.

Valdivia A[7,1], Racordon D[7], Mingo G[7,1], Bravo M L[7,2], Sandoval A[7,1], González A[3], Retamal C[3], Cuello M[7], Sanchez B[4], Nualart F[5], Corvalán A[7,2,1], **Owen G[7,6,2,1]**, [1]Advanced Center for Chronic Diseases (ACCDiS) Pontificia Universidad Católica de Chile.[2]Center UC Investigation in Oncology Pontificia Universidad Católica de Chile.[3]School of Medicine, Universidad San Sebastian, Santiago, Chile, & Center for Ageing and Regeneration (CARE) Pontificia Universidad Católica de Chile.[4]Institute of Physics Pontificia Universidad Católica de Chile, Santiago, Chile.[5]Faculty of Sciences Universidad de Concepcion, Chile.[6]Millennium Institute on Immunology and Immunotherapy Pontificia Universidad Católica de Chile.[7]Faculties of Biological Sciences & Medicine Pontificia Universidad Católica de Chile.

The presence of Vasculogenic mimicry (VM), the establishment of an alternative perfusion pathway in the absence of endothelial cells, strongly correlates with poor patient survival. Despite many publications claiming to demonstrate VM *in vitro*, controversy still surrounds the existence of an assay showing true hollow channels. Herein, we provide the most convincing evidence to date of the formation of functional hollow channels in *in vitro* cancer cultures. Fluorescence confocal microscopy, X-ray microtomography 3D-reconstruction and luminescent dye microinjection conclusively demonstrate functional glycoprotein-rich lined hollow structures surrounded by an outer cancer cell layer. We present evidence that these structures are triggered by an element of the extracellular matrix and require a signaling pathway independent to that used in angiogenesis. This assay may aid the design and testing of future VM-blocking anti-cancer therapies.

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Dr. Gareth Owen is an associate professor of Faculty of Biological Sciences and Faculty of Medicine at Pontificia Universidad Católica de Chile. He received his undergraduate degree from King's College London and his Doctorate in Biochemistry at the Royal Postgraduate Medical School, Hammersmith Hospital, Imperial College London. He completed postdoctoral degrees at the University of Colorado, E.U.U and later at the Cancer Research Institute (ICR) in London, UK. His area of major interest is cancer research, with an extensive list of publications and 14 graduate doctoral students (www.labowen.cl). Dr. Owen is one of the founders and one of the principal researchers of the Biomedical Research Consortium of Chile (BMRC, www.bmrc.cl), a member of the commission of the Clinical Oncology Research Group of Chile (GOCCHI, www.gocchi .org), deputy director of the UC Center for Research in Oncology (CITO) and leader of the Research Division for the National Cancer Forum of Chile (FNC, www.foronacionaldecancer.cl)

Gallbladder cancer. Molecular pathology and preclinical studies with potential impact on patient management.

Roa J C[1], [1]Pathology, Medicine, Pontificia Universidad Católica de Chile.

Gallbladder cancer is the second leading cause of cancer death in women over 40 years in Chile.Characteristically is a highly lethal disease with frequent late diagnosis, intrinsically linked to the presence of gallstones, has few therapeutic tools when diagnosed in advanced stages. There several clinical problems in the diagnosis and management of patients with gallbladder cancer that require novel approaches based on the basic mechanisms underlying this disease that produce and modulate the biological behavior of this tumor.

During the presentation different issues that allow to build new research lines of study of gallbladder cancer, including identification of biomarkers of genetic predisposition, early diagnosis, prognosis, prediction and selection of therapy will be delivered. Proper use and access to institutional Bioarchives and Biobank, and the use of *In vitro* and *in vivo* models and clinical trials have enabled to generate relevant information on the best classification of preneoplastic and neoplastic of the gallbladder. Identification of metabolic pathways altered (AKT mTOR, HSP90, Hippo) that are susceptible to become new specific targeted therapies as well as the proper monitoring of the disease progression and response to treatment by new platforms such as liquid biopsy (exosomes circulating tumoral cells and free cell DNA) will be highlighted.

Advancer Center for Chronic Diseases - ACCDIS.

Millennium Institute on Immunology and Immunotherapy (P09- 016-F). FONDECYT Grant 1170893.

Dr. Juan Carlos Roa studied medicine at the University of La Frontera and specialized in Surgical Pathology at Pontificia Universidad Católica de Chile (PUC). He did a Fellowship in Molecular Biology at Louisiana State University and a Master in Molecular Oncology in Spain CNIO. In 2010 he was appointed Professor at the University of La Frontera, being Director of Postgraduate Research and director of the Laboratory of Molecular Pathology. Dr. Roa joined the PUC in 2012 and since then has been teaching in undergraduate courses and Functional Morphological Pathology in Medicine and Dentistry and the medical sciences PhD Program in medical Sciences and applied cellular and molecular biology at PUC and Universidad de La Frontera respectively. He is part of the staff of the residence program in Gastroenterology, Pathology and Medical Oncology being tutor of several PhD students and Postdoctoral training programs in molecular Oncology. He has participated in numerous research projects FONDECYT, FONDEQUIP, CORFO as a Principal investigator and associate researcher at FONDEF, FONDAP and National Cancer Insitute (USA) projects. He has published more than 215 articles in peer reviewed national and international journals, also author of 13 book chapters. Dr. Roa, he was appointed Chairman of the Department of Pathology in October 2012 and Professor of pathology in 2015 at Pontificia Universidad Católica de Chile. He coordinated the creation of the Biobank Medical School who currently heads and is also the head of laboratory of Molecular Pathology for gallbladder Cancer Research.

A New Melanoma Vaccine Based on Conditioned Tumor Cell Lysates and Adjuvants Inhibits Tumor Growth.

Salazar F[1], [1]Inmunología, Facultad de Medicina, Universidad de Chile.

Clinical strategies using immune-checkpoint blockers, such as anti-CTLA4 or anti-PD1 antibodies, have demonstrated durable survival benefits in patients with melanoma and other tumors. Nevertheless, an important percentageof treated patients remain refractory, suggesting that combination with other kind of active immunization may impact responses rate. In this context, cancer vaccines become again a complementary alternative as cancer treatment. The optimal delivery of antigens (Ags) and the use of adequate adjuvants are crucial for vaccine success. Here, a prototype of a generic therapeutic vaccine for the treatment of malignant melanoma named TRIMELVax™ was tested in an experimental model. This vaccine is based on conditioned melanoma allogeneic tumor lysates (TRIMEL) combined with a specific adjuvant. The vaccine is intended to directly activate the immune response against tumor *in vivo*, inhibiting its growth. TRIMELVax was evaluated, in terms of safety and efficacy in C57Bl/6 murine model of melanoma. In short, immunocompetent mice was vaccinated with three doses of TRIMELVax, and then challenged with B16 cells. Alternative, immunization was also tested therapeutically in tumor bearing mice. Our results showed that only TRIMELVax was capable to reduce the occurrence of tumor, inhibiting tumor growth in immunized mice. In contrast, the use of tumor lysate, or adjuvant alone did not impact tumor growth. This response was associated to CD8+ T cells mediated response and antibody production. Immune-checkpoint blockers therapy may be strongly potentiated by the combination with TRIMELVax, which need to be proved in future clinical trials.

(Sponsored by Financed By Grants FONDECYT 1171213; FONDEF ID16I10148 And MIII P09/016-F.)

Dr. Flavio Salazar Onfray is a biologist from the University of Uppsala, Sweden, and got his PhD at the Karolinska Institute. In 1999 he got a position at the Disciplinary Program of Immunology at the Institute of Biomedical Sciences, in the Faculty of Medicine, University of Chile. Dr. Salazar interest has been focused in several aspects of tumor immunology, from basic research to clinical trials. Dr. Salazar has identified and characterized new melanoma antigens British J Immunol. (2005); IOVPS (2007). During the last decade, Dr. Salazar has lead a multidisciplinary group and performed the first Phase I and Phase I/II clinical trials in Chile of immunotherapy based on dendritic cells for the treatment of melanoma and also for prostate cancer. The results of these clinical studies have been published in high impact international journals such as Clin. Exp. Immunol. (2005); J. Clin. Oncol. (2008); Clin. Cancer Res (2011) and Cancer Immunol. Immunother. (2012) Brit J cancer (2013). He and his group invented an original method to produce dendritic cells ex vivo protected by an international patent (PCT/EP2008/062909). Recently, he also described gap junction (GJ) interaction between human immune cells and tumors (J. Immunol. 2007, J. Immunol. 2011, 2014). Dr. Salazar has contributed directly to the formation of 11 Phd, and several MSc. Since 2005, Dr. Salazar (Sub director) together with Dr Kalergis (Director) leads the Institute Millennium of Immunology and Immunotherapy, where new group leaders have been formed starting new immunology groups. Dr. Salazar has pre and post graduated teaching responsibilities at the University of Chile, coordinating several courses in immunology and oncology. He has participated in several scientific societies and he is the former President of the Chilean Society of Immunology and the past President of the Latin American association of Immunology Societies (ALAI). He obtained several research grants from national and international agencies (Cancerfonden Sweden; University of Chile; FONDECYT Chile, Fondef Chile, CORFO and Millennium) and he has become the leader of several R&D projects and founds also two spin off companies, Oncobiomed in 2002, dedicated to cellular immunotherapy technology transfer and Bionex in 2007, dedicated to advice basic scientist in the design and writing of R&D applications. In 2007 and 2008, he was nominated for the innovation AVONNI price by the Forum for the innovation from the Ministry of Economy. In 2009 he was part of the Chilean President delegation during the state visit to India. From June 2014 he became Vice President of Research and Development at the University of Chile.

Dra. Claudia Quezada is Biochemist from Universidad Austral de Chile and PhD in Molecular Biosciences at Universidad Andrés Bello. She is currently Associate Professor and Principal Investigator of the Molecular Pathology Laboratory of Sciences Faculty at Universidad Austral de Chile. Her research line is related to the study of multiple drug resistance and adenosine signaling in stem cells of Glioblastoma multiforme. The year 2013 won the national prize for research University "Cure and Prevention of Cancer". She has published in major journals such as Medicinal Aspects of Medicine, Oncotarget, Journal of Neuro-Oncology, J. Cell Physiology, etc., showing the role of various modulators of drug resistance in the treatment of glioblastoma. She has executed 20 international and national grants as the principal investigator or coinvestigator (FONDECYT, FONDEQUIP, FONDEF, ANILLO, FIDECNO). She has directed 17 graduate and 5 undergraduate theses, is faculty member of several PhD programs, as well as national and international societies.

Dra. Veronica Burzio is a Senior Investigator at Fundación Ciencia & Vida and Andes Biotechnologies, and Assistant Professor at Universidad Andrés Bello in Santiago, Chile. She is a Biochemistry graduate from the Universidad Austral de Chile and PhD in Molecular and Cell Biology and Neuroscience from the Universidad de Chile. For several years Dr. Burzio has done research in the field of molecular bases of cancer, including cell signaling and molecular biology and has published in several peer-reviewed journals. She is also author of several International Patents and Patent Applications in the field of cancer. At present she is investigating the function of the novel family of the long noncoding mitochondrial RNAs in tumorigenesis, its utility as targets for cancer therapy, as well as the molecular mechanisms underlying selective cell death and inhibition of tumorigenical traits of tumor cells, induced by knockdown of members of this family of transcripts.

Symposium

Structural Biology-Protein Homeostasis Sbbq Brazil

Chairs: Maximiliano Figueroa- Christian A.M. Wilson

A comparative analysis of protein structures - studying how proteins structural differences influence on their function.

Neshich G[1], [1]Computational Biology Research Group - Embrapa Agricultural Informatics Embrapa.

A new method of comparing structurally aligned proteins will be presented. Such method consists of observing correspondingly equivalent loci in such alignment but instead of using configurational differences as a metric for quantifying divergence among compared structures, we now use a list of descriptors and their values in order to elaborate on variations that might be reflected on functional changes.
Consequently, by using protein structural and physical-chemical parameters/descriptors, we are now capable of uniquely describing and identifying functional districts in proteins - a case study that would be presented: protein interfaces and differences in functionally observable protein interactions!
Embrapa-Fapesp- SBBq.

Goran Neshich, Ph.D., is leader of the Computational Biology Research Group (CBRG) at the Brazilian Agricultural Research Corporation (EMBRAPA), National Agricultural Information Technology Research Center (CNPTIA), Campinas - SP, Brazil. Neshich started his work in structural bioinformatics while studying structure and function relationship of the photosynthetic reaction center during his graduate work done with Don DeVault at the University of Illinois at Urbana-Champaign (1983-1988). He conducted his postdoctoral research with Barry Honig at Columbia University (1997-1998). Neshich is the principal author of STING suite of programs (with the current version being BlueStarSTING), and STING_DB. STING is a popular database and visualization tool providing the largest collection of physicochemical parameters that describe protein structure, stability, function, and interaction with other macromolecules. STING received approximately 17 million accesses since 1998. Neshich has published 54 papers in indexed scientific journals and 125 abstracts at the meetings and congresses. He co-authored 4 patents and conceptualized 51 software packages and is the author of the First databank from Latin America which made it to the compendium of the Nucleic Acid Research - Database Issue: the STING_DB. He gave more than 100 seminars and talks at the congresses and research institutions at 16 countries from 5 continents. He was co-adviser for 19 PhD theses, 6 Master's degree theses and 6 scientific initiation works as well as for 3 postdocs in the area of Computational Biology. Currently, Neshich is advising and co-advising theses work of two students: two (2) PhD. From 1990 through 2017 he coordinated 24 research projects and co-participated in other 3 projects, with financial input under his coordination of approximately US$1.7 Million. Neshich's latest initiative is further developing the STING RDB and by using this unique resource / tool, advance our understanding of the relationship between sequence, structure and function of proteins, and consequently, to be able to describe in more details protein - protein and protein-substrate interaction. His special interest now is in high throughput screening and rational drug and agrochemical design. More about his research may be found at www.cbi.cnptia.embrapa.br. Neshich chaired a session at the meeting in Angra dos Reis - RJ, held in November 2004, where the Brazilian Association for Computational Biology and Bioinformatics (AB3C) has been inaugurated. He was a member of the Board of Directors of the International Society for Computational Biology (ISCB) from 2003 to 2005 and the Intelligent Systems in Molecular Biology (ISMB) 2006 Conference Chair in Fortaleza, Brazil. Neshich served as the member of the Excutive Borad of EMBnet from 2012 to 2015 when he resigned. In April of 2012 Neshich orginized São Paulo Shool of Advanced Sciences: Advanced Topics in Computational Biology - Agrochemical and Drug Design (ESPCA_CBADD). Currently Neshich is the leader of the Structural Computational Biology Research Group at Embrapa.

Understanding natural proteins through artificial proteins: the Octarellins as a model.

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

The central dogma in structural biology is that the tertiary structure of a protein is defined by its primary structure. Defining the fold of a protein from its sequence is called the protein folding problem. The "inverse" protein folding problem rises then as given a tertiary structure, defining a sequence(s) that folds into this given structure. Solving the inverse protein folding problem has huge impact in protein engineering, because it represents, first, a demonstration of a full knowledge about protein structure and its interpretation through physical and mathematical models; and second, using this information we can create new proteins, exploring new folds and define synthetic enzymatic reactions. This fascinating statement, however, is far to be achieved. Several groups around the world are trying to solve the inverse protein folding problem, with only few examples of success.

The Octarellin project born in the 90s with the final goal of solve the inverse protein folding problem using as target the (βα)8 fold, or better known as Tim-barrel. This fold is widely found in nature: 10% of the proteins are able to adopt it, harvesting 5 of the 6biochemical reactions. Then, the TIM-barrel fold is an ideal scaffold to try to define artificial sequences able to fold as it. This lecture will summarize our different approaches to design proteins, and how these have led to create different protein generations, to finally discuss what we have learned from our still increasing experience and how this knowledge can be extrapolated to design different proteins.

Dr Maximiliano Figueroa (36) obtained the professional title of Biochemist in 2005 at the University of Concepción. During his undergraduate thesis, directed by Dr Marta Bunster, he had a first approach with protein structures, developing an *in silico* docking model of phycobiliproteins, fluorescent proteins present in algae and cyanobacterias, to create an antenna which explained, structurally, the light conduction in this system. In 2005 he started his PhD in Biological Science at the University of Concepción, under the co-direction of Dr José Martínez-Oyanedel and Dr Juan Olate. During his PhD thesis, Dr Figueroa worked in understand structurally the many functions of the protein Ric-8. He managed to propose the first structural model for this protein, supported by biophysical data. To accomplish this purpose, he stayed few months in the laboratory of Patricia Babbitt, University of California at San Francisco. Dr Figueroa finished his PhD in 2010, and his thesis was recognized as one of the most outstanding PhD thesis of 2010 by "Red Cruz del Sur", a Chilean universities network. The same year he started a postdoctoral training in the laboratory of Molecular Biomimetics and Protein Engineering, leaded by Dr Joseph Martial and Dr Cécile Van de Weerdt (GIGA-R Center, Université de Liège, Belgium). During his postdoctoal training, Dr Figueroa worked in two main projects: developing protein model systems to study mass transport in protein crystallization under micro-gravity conditions, and design and characterization of artificial proteins with (b/a)8 fold, named Octarellins. This postdoctoral training was funding by two academic excellence fellowships, Wallonie-Bruxelles International organization and Belgian Science Policy, and by the European Space Agency. During his postdoc, he could crystallize and determine for the first time the x-ray structure of an artificial protein of more than 200 amino acids. Moreover, he managed to establish collaboration regarding structural biology fields with Dr Philippe Minard and Dr Dominique Durand (Université de Paris Sud, France), Dr Dominique Maes and Dr Mike Sleutel (Vrije Universiteit Brussels, Belgium), Dr Jens Meiler (Vanderbilt University, USA), and Dr André Matagne (Université de Liège, Belgium). The scientific contributions of Dr Figueroa have been published in recognized journals of the field of structural biology, and he has been invited to give lectures in Europe, Chile and Japan. In 2016 Dr Figueroa is engaged by Universidad de Concepción, where he is currently assistant professor to boost the structural biology field in the Department of Biochemistry and Molecular Biology. Dr Figueroa has teaching responsibilities in under and post graduate courses, participating in Biochemistry, Protein Engineering, and Biophysics courses.

Structural and functional analysis of the systems for sulfur uptake and assimilation in the phytopathogenic bacterium *Xanthomonas citri.*

Pereira C[1], Sampaio A[2], Fessel M[3], **Balan A[4]**, [1]Genetics and Molecular Biology, Institute of Biology, Universidade Estadual de Campinas.[2]Biotechnology, Institute of Biomedical Sciences, University of São Paulo.[3]Laboratório Nacional de Biociências Centro de Pesquisas em Energia e Materiais.[4]Microbiology, Institute of Biomedical Sciences, University of São Paulo.

The *Xanthomonas citri* pv. *citri* (*X. citri*) is a phytopathogenic bacterium that infects different species of citrus plants causing the canker disease. The adaptation to different habitats is related to the ability of the cells to metabolize and to assimilate diverse compounds, including sulfur, an essential element for all organisms. In *Escherichia coli,* the necessary sulfur can be obtained by a set of proteins whose genes belong to the *cys* regulon. Although the *cys* regulon proteins and their importance have been described in many other bacteria, there are no data related to these proteins in *X. citri* or in the *Xanthomonas* genus. In this work, using functional and structural approaches we showed the presence of at least four distinct ATP-Binding Cassette transporter systems for sulfur assimilation in *X. citri* and evidenced their importance during in vitro and in vivo growth. From the four systems, three are dedicated to uptake of sulfonates and one for sulfate. A comparative analysis of the full transporters revealed significant differences in the ligand-binding pocket of the periplasmic-binding proteins, which are responsible for specificity and affinity of the transport, and related enzymes. In addition, deletion of genes encoding the periplasmic components in *X. citri* revealed they also have a role in the xantham gum production and pathogenesis of the bacterium. Altogether, these results serve as a foundation for further studies aimed to understanding the relevance of sulfur in growth, virulence and pathogenesis of *X. citri* and related bacteria.

Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Prof. Andrea Balan has a BA in Biological Sciences by the State University of Campinas (Unicamp, 1990), Master in Biotechnology (1994) and PhD in Microbiology (1999), both developed at the University of São Paulo – USP, Brazil. Her work was focused on the heterogeneous expression of proteins in yeast and bacteria aiming the production of biotechnological products. Dr. Balan started a post-doc in Structural Biology of Proteins at the Institute of Biomedical Sciences of the University of São Paulo working with ATP-Binding Cassette transporters from pathogenic bacteria (2000-2004), continued at the Brazilian National Laboratory of Biosciences – LNBio (2008), Campinas, and then spent 3 years at the California Institute of Technology (Pasadena, USA) and the University of Cambridge (Cambridge, England), always working on Structure and Function of Proteins and protein-protein interactions. In 2010 she became research leader at the LNBio in Campinas and in 2014 she became Professor at the Department of Microbiology at the University of São Paulo in São Paulo. His area of action involves mainly the structural and functional characterization of transport proteins and their role in the mechanisms of virulence, pathogenesis and drug resistance. Also works with the structural characterization and engineering of proteins of biotechnological interest, including membrane proteins involved with the exclusion of drugs.

Dynamics of membrane localization of the protein translocation motor SecA in E. coli.

Driessen A[1], [1] University of Groningen.

Protein translocation across the bacterial cytoplasmic membrane is an essential process catalyzed predominantly by the Sec translocase. This system consists of the membrane-embedded protein-conducting channel SecYEG, the motor ATPase SecA, and the heterotrimeric SecDFyajC membrane protein complex. Anionic lipids are essential for SecA activity and the N terminus of SecA is capable of penetrating the lipid bilayer. However, the role of lipid binding has remained elusive. By employing differently sized nanodiscs reconstituted with single SecYEG complexes and comprising varying amounts of lipids, we establish that SecA gains access to the SecYEG complex via a lipid-bound intermediate state, whereas acidic phospholipids allosterically activate SecA for ATP-dependent protein translocation. We further examined the dynamics of the cellular location of SecA in Escherichia coli cells using super resolution microscopy. Chromosomally encoded SecA fused to a fluorescent reporter is predominantly associated with and evenly distributed along the cytoplasmic membrane as a homodimeric protein with only a minor fraction in the cytosol. SecA frequently localizes to short-lived foci at the membrane and diffuses as three distinct but interconvertible populations with diffusion constants that correspond to a peripheral membrane associated form, a membrane integral, likely SecYEG associated state, and a very slow diffusing population. These data support a model in which dimeric SecA diffuses along the membrane surface to gain access to the SecYEG translocon.

Arnold Driessen obtained his PhD (*cum laude* degree in 1987) with Prof. W. Konings at the University of Groningen in the Netherlands where he developed a generic method for the *in vitro* energization of membranes derived from fermentative bacteria. This technical advance allows detailed studies on transport processes in membranes of fermentative and strictly anaerobic bacteria, as well as in plasma membranes of yeast, plants, and even mammalian cells. He then went for a Postdoctoral stay at UCLA, USA in the group of Prof. W. Wickner (1989-1990) where he reconstituted as the first, the bacterial protein translocation system with purified components thereby obtaining detailed insights in the mechanism and energetics of protein transport. This work defined the minimal set of components required for protein translocation and the identification of the translocase, a complex of a peripheral membrane bound motor protein SecA and the membrane-embedded protein-conducting channel SecYEG. The work belongs to one of the mostly cited papers in the field and has been leading for later discoveries on the translocon in the mammalian endoplasmic reticulum membrane. He then returned to the University of Groningen where he established a group that studies various aspects of membrane biology, transport and protein translocation. Since 2002, Driessen is full Professor at the University of Groningen. His research has a strong multidisciplinary character including single molecule physics, synthetic-organic chemistry, and structural biology. Driessen has published more than 440 manuscript in peer-reviewed journals with a high citation record (>24,000) and a very high *h*-factor (80). He holds 11 patents. Driessen is frequently plenary speaker at the international Gordon, FEBS and EMBO conference, and organized several of these conferences. In 1988, Prof. Driessen obtained the Kluyver Award of the Dutch Society of Microbiology, and in 1993, he was awarded the Anniversary Price of the Society for Biological Chemistry Federation of the European Biochemical societies (FEBS). Driessen has been scientific director of the Groningen Biomolecular Sciences and Biotechnology Institute (GBB) at the University of Groningen, and was member of the board of Life Sciences of the Netherlands Organization of Fundamental Research (NWO). He is a member of the Netherlands Academy of Sciences and Arts (KNAW), flagship leader in the public-private research programme BE-BASIC, and scientific advisory board member of various institutes and organizations.

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. His laboratory focuses in single molecule manipulation of biomolecules. This area is a new field of research and allows studying the effect of the forces on the structure of proteins and the concomitant changes in their function. It also permits to determine the forces and torques developed in the course of the mechanochemical conversion in molecular motors. Inside the cell, mechanical forces are produced in molecular processes as diverse as transcription, replication, translation, chromosomal segregation, protein unfolding, translocation of proteins across the membranes and cellular movement. Now, their work is focused in determining the importance of the force associated to the domain movements of BiP (immunoglobulin heavy-chain binding protein) protein during protein translocation in the ER in a collaboration with Randy Schekman´s group, also focusing in the kinetic properties of BiP and in the conformational changes that occur during its ATPase cycle, as it is working in the translocation process. Dr. Wilson lab has assembled the first optical tweezers instrument to measure force in individual molecules in the country.

Symposium

The role of DNA viruses and encoded oncoproteins in human carcinogenesis
Chair: Francisco Aguayo

The hepatitis B viral protein HBx is required to establish an active hepatitis B viral chromatin state.

Loyola A[1], Alvarez F[1], Hernández S[1], Garrido D[1], Villanueva R[1], [1]Laboratory of Epigenetics and Chromatin Fundación Ciencia & Vida.

With about 350 million people chronically infected around the world hepatitis B is a major health problem. Template for progeny HBV synthesis is the viral genome, organized as a minichromosome (cccDNA) inside the hepatocyte nucleus. How viral cccDNA gene expression is regulated by its chromatin structure; and more importantly, how the modulation of this structure impacts on viral gene expression remains elusive. We will discuss the role of the viral protein HBx on the regulation of the viral cccDNA chromatin, mainly as a recruiter of histone modifying enzymes. In addition, we will present evidence that HBx expression is regulated by alternative translation initiation, and that one of the HBx isoforms is sufficient to elicit HBV transcriptional activation. Our results shed light on the mechanisms of HBV regulation mediated by the cccDNA chromatin structure, offering new therapeutic targets to develop drugs for the treatment of chronically infected HBV patients.

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ANILLO ACT1119, BASAL Project PFB16, FONDECYT 1120170.

Dr. Alejandra Loyola is a Biochemist from Universidad de Chile. In 1997, she entered to the PhD program of Rutgers University in New Jersey, USA. Her thesis work, performed in the lab of Dr. Danny Reinberg, was focused on the characterization of RSF, a chromatin assembly factor identified in the Reinberg's lab. After graduating in 2003, Dr. Loyola moved to the Institut Curie, in Paris, France, and worked at the lab of Dr. Geneviève Almouzni for her post-doctoral training. She investigated post-translational modifications occurring on histone H3 variants. During those years of training, she was awarded with an Institut Curie fellowship and then with an EMBO fellowship. In 2006 Dr Loyola started her second post-doctoral training at the MD Anderson Cancer Center in Houston, Texas, USA, in the lab of Dr. Sharon Dent. Her work focused on the enzymes involved in histone post-translational modifications. She was awarded with an Odyssey fellowship. In 2008 she established her own lab at Fundación Ciencia & Vida, where she is Head of the Epigenetics and Chromatin Lab. Her research focuses on understanding the mechanisms by which chromatin and its modifications regulate cellular processes. Her lab has three main areas of investigation: the analysis of post-translational modifications of histones before they are incorporated into chromatin, the role of the viral chromatin in the replication of the Hepatitis B Virus, and the characterization of the epigenetic modifications during the differentiation of T lymphocytes.

Epstein-Barr virus and gastric cancer.

Corvalan A[1], [1]Advanced Center for Chronic Diseases, Medicine, Pontificia Universidad Católica de Chile.

Gastric cancer is a leading cause of cancer deaths. Comprehensive molecular evaluation of primary gastric carcinomas by The Cancer Genome Atlas (TCGA), have proposed molecular classification dividing gastric cancer into four subtypes: Epstein-Barr virus-associated gastric carcinoma (EBVaGC), microsatellite unstable tumours, genomically stable tumours, and chromosomal instability gastric tumors. The EBVaGC subtype is more prevalent in the Americas than any other regions in the world. This type of gastric cancer is also more frequent in males and portray predominantly diffuse-type histology. EBERs, EBNAs, BARTs and LMP are the highest expressed genes. EBVaGC cases harbor exclusively the western genotype (subtype D and kept Xho I site), suggesting a disrupted co-evolution between the pathogen and its host. EBVaGC display recurrent PIK3CA mutations, extreme DNA hypermethylation, and amplification of JAK2, CD274 (PD-L1) and PDCD1LG2 (PD-L2). Environmental conditions include negative associations with being firstborn child and positive association with smoking. The role of H. pylori coinfection is not conclusive for variant of gastric cancer. This presentation is a contribution to the rapidly unfolding story of this novel molecular subtype of gastric cancer that has transpired over the last 20 years until recognition by the TCGA.

Conicty-Fondap 15130011, FONDECYT 1151411 and FONDECYT 1161219.

Dr. Alejandro Corvalán is Medical Doctor and Master in Sciences from University of Chile in 1985 and 2006. Clinical and research training in Molecular Pathology at Mount Sinai School of Medicine in New York and Tokyo Medical and Dental School of Medicine in 1992 and 2001. Currently Associate Professor at Pontificia Universidad Católica de Chile and Principal Investigator of the Advanced Center for Chronic Diseases (ACCDiS). Academic activities include translational research as well as postgraduate education in Oncology. His research focus in clinical and molecular associations of gastric cancer. Through this approach he discovers a high association of the Epstein-Barr Virus (EBV) as well as the presence of unique strain of EBV in gastric cancer. In addition, he has strong interest in hereditary tumors as well as the role of epigenetics in precursor lesions of gastric cancer. These discoveries not only contribute to the understanding of the pathogenesis of these neoplasms but also act as potential biomarkers for non-invasive diagnosis, monitoring treatment response and developing new treatments.

The role of high-risk human papillomavirus in carcinogenesis: Signaling pathways and interaction with environmental carcinogens.

Carrillo D, Muñoz J, Tapia J, Corvalán A[1], Boccardo E[2], **Aguayo F[3]**, [1]Advanced Center for Chronic Diseases (ACCDiS), Medicina, Pontificia Universidad Católica de Chile.[2]Department of Microbiology, Medicine, Enrique Boccardo.[3]Departamento de Oncología Básico Clínica, Medicina, Universidad de Chile.

High-risk human papillomavirus (HR-HPV) infection is etiologically related to cervix-uterine, anogenital and head and neck carcinogenesis. However, HR-HPV infection is not a sufficient condition for cancer development. The hallmark of HR-HPV-related carcinogenesis is E6 and E7 oncogene overexpression. Thus, the first focus of research is the characterization of epithelial oral and cervical cancer cells that express HR-HPV E6 and E7 oncoproteins. Transcriptomic assay using DNA microarrays revealed that PIR gene expression was detected in oral cells in a HR-HPV E6/E7 dependent manner. In addition, PIR was overexpressed in HPV-positive SiHa and CasKi cells whereas was undetectable in HPV-negative C33A cells. The PIR expression was dependent on functional HR-HPV E6 and E7 oncoproteins even though the E7 oncoprotein had higher activity to induce PIR overexpression in comparison to E6. In addition, using a siRNA for PIR silencing in oral cells ectopically expressing HR-HPV E6/E7, there was a significant increase of E-cadherin transcripts and a decrease of Slug, Zeb and Snail transcripts, suggesting that HR- HPV-induced PIR overexpression is involved in epithelial-mesenchymal (EMT) transition. Finally, it was found that EGFR activation is important for E7- mediated PIR overexpression. It can be concluded that PIR gene expression is highly dependent on the expression of HR-HPV oncoproteins and is important for EMT regulation. The second focus of research is the cooperation between E6/ E7 oncoproteins and environmental carcinogens. Epithelial cells were exposed to both HR-E6/E7 oncoproteins and cigarette smoke components. Some signaling pathways important for such cooperation have been identified.

This study was supported by FONDECYT Grants 1161219 to FA, 1160889 to JT and CONICYT- FONDAP-15130011.

Dr. Francisco Aguayo obtained his Bachelor degree in Biochemistry at University of Chile in 1992 and received a Ph.D. in Medical Sciences with mention in Persistent and Oncogenic viruses at Faculty of Medicine, University of Kagoshima, Japan in 2007. His doctoral dissertation was about the role of high-risk human papillomavirus (HR-HPV) in extragenital tumors. Currently he is Associate Professor at Department of Basic and Clinical Oncology, Faculty of Medicine, University of Chile and Associate Researcher at Advanced Center for Chronic Diseases (ACCDiS). He has postdoctoral experience in Europe and U.S. working in different models of viral-induced carcinogenesis. Since the mere expression of viral oncoproteins is not a sufficient condition for carcinogenesis, his research interests are signaling pathways related to interactions between viral oncoproteins and environmental carcinogens. Now he is researching the mechanisms involved in a cooperation between human papillomavirus (HPV) E6 and E7 oncoproteins and tobacco smoke for human carcinogenesis using different models of epithelial and tumor cells. In addition, he is interested in Epstein-Barr virus (EBV)-mediated carcinogenesis including gastric cancer and specially the role of EBNA-1 oncoprotein.

Dna Repair Machinery Plays A Critical Role In The Survival Of Cells Transformed With Human Papillomavirus.

Boccardo E[1], [1]Microbiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo.

Infection with some mucosal human papillomavirus (HPV) types is etiologically associated with almost all cases of cervical cancer and with a significant fraction of vaginal, vulvar, anal, penile and head and neck carcinomas. DNA repair machinery is essential in some steps of life cycle and crucial for tumor cells survival. In the present study we silenced over a hundred genes involved in DNA Repair pathways, to identify those essential HPV-transformed cells survival. We identified three genes, *ATM*, *CHEK2* and *BRCA1*, which down-regulation selectively affects the proliferation of cervical cancer derived cell lines SiHa and HeLa without altering normal primary human keratinocytes (PHK) growth. Furthermore, silencing or chemical inhibition of these genes reduced the clonogenic and proliferative capacity of cervical cancer derived cells. We also show that this effect is paralleled by an increase in the number of hypodiploid cells. Experiments conducted using PHK transduced with HPV16 oncogenes show that the effect of ATM, CHEK2 and BRCA1 silencing depends on the expression of E6 oncogene and on its ability to induce p53 degradation. Importantly, we provide evidence that treatment of p53-deficient cells from different origins with ATM/CHEK2 inhibitors downregulates cell proliferation. Our results show that inhibition of components of the ATM signaling axis reduces p53-deficient cells proliferation potential, suggesting the existence of a synthetic lethal association between CHEK2 and p53. These preclinical observations may prove relevant for the development of alternative therapeutic strategies due to the high frequency of p53 mutations in human tumors.

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Dr. Enrique Boccardo is a Molecular Biologist with over 20 years of experience in academia. Dr. Boccardo was graduated from the Faculty of Sciences of the University de la República, Uruguay, in 1994. He completed his MSc in Cellular and Molecular Biology in 1997 at the University de la República, Uruguay. He completed his PhD in Biochemistry in 2002 at the University of São Paulo. He has served as a scientific researcher at the Ludwig Institute for Cancer Research, São Paulo branch, during 2002-2010. Since 2010 he is a Professor and Head of the Laboratory of Oncovirology in the Department of Microbiology of the Institute of Biomedical Sciences at the University of São Paulo. Dr. Boccardo is member of Latinoamerican Academy of Sciences (ACAL) and Coordinator of the Scientific Committee of the National Institute of Sciences and Technology of HPV related diseases (INCT-HPV). In 1998 he was awarded with National Academy of Medicine of Uruguay award. Dr. Boccardo has written more than 30 peer reviewed publications and book chapters. Enrique Boccardo leads nationally funded research projects with a focus on the mechanisms of human papillomavirus mediated cell transformation, genome instability induction, immune evasion establishment, and in the development of epithelial organotypic cultures. Besides, he is involved in projects that aim the identification of prognosis/progression molecular markers for HPV associated pathologies.

Symposium

Cellular Quality Control & Communication in Health & Disease
Chairs: Sergio Lavandero-Andrew Quest

ER-mitochondria communication in cardiac pathology.

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Repetitive, calcium-mediated contractile activity renders cardiomyocytes critically dependent on a sustained energy supply and adequate calcium buffering, both of which are provided by mitochondria. Physical and functional communication between ER and mitochondria and balanced mitochondrial dynamics seem to have a critical role for optimal calcium transfer to mitochondria, which is crucial in calcium homeostasis and mitochondrial metabolism in cardiomyocytes. Moreover, mitochondrial dysfunction has been associated with myocardial damage. Therefore, ER-mitochondria coupling and mitochondrial dynamics are now viewed as relevant factors in the pathogenesis of some cardiac diseases, including coronary artery disease, heart failure, and pulmonary arterial hypertension. In this talk, we will provide the evidence related to the role of ER-mitochondria communication in cardiac muscle physiology, with a focus on how perturbations contribute to the pathogenesis of cardiac diseases

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Sergio Lavandero, PhD is Full Professor with positions in two academic units (Faculty of Chemical & Pharmaceutical Sciences & Faculty of Medicine) of the Universidad de Chile. Also Dr. Lavandero holds an Adjunct Professor position in the Cardiology Division, Department of Internal Medicine, University of Texas Southwestern Medical Center (Dallas, Texas). He is author of 227 publications in ISI journals (h index: 49, 13,170 citations, Google Scholar). He is currently Director and Principal Investigator in the FONDAP Advanced Center for Chronic Diseases (2014-2024) and Principal Investigator in the Center for Molecular Studies of the Cell (CEMC). He is Associate Editor of Circulation and member of the editorial boards of American Journal of Physiology (Endocrinology & Metabolism section), Cell Death & Diseases and BBA Molecular Basis of Diseases, He is *ad hoc* reviewer in many international journals and international scientific research funding institutions and an active member in several national and international scientific societies. He is also member of the Chilean Academy of Sciences. His research lines are: a) Mitochondrial dynamics and cardiac diseases. b) Regulation of mitochondrial dynamics and metabolism by insulin in the heart and skeletal muscle. c) ERmitochondria communication. d) IGF-1 receptor signaling pathways in the heart. e) Primary cilia and heart function.

Protein disulfide isomerases: new players in redox signaling and homeostasis.

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Protein disulfide isomerases are thiol oxidoreductase chaperones from the thioredoxin superfamily with the canonical function of redox protein folding catalysis at the endoplasmic reticulum (ER). PDIA1, the family prototype, merges and cooperates with >20 family members to exert thiol oxidation/reduction and isomerization, as well as chaperone effects. PDI(s) also regulate thiol-disulfide switches at the cell surface, extracellular milieu and possibly cytosol and nucleus. In vascular cells, PDIA1 is required for agonist-triggered activation of Nox-family NADPH oxidases and physiological readouts such as cell migration and phenotype switches. PDI(s) exert crucial roles in thiol redox regulation of thrombosis and platelet activation via several targets that include integrins. Also, PDIA1 converges with cytoskeletal regulation beyond Nox and integrin regulation. Effects include thiol switches involving oxidation, reduction and isomerization, among others, leading to protein oligomerization or (de)activation. PDI effects may be orchestrated by expression levels as well as post-translational modifications. PDI is sensitive to redox environment, but the "all-in-one" organization of its redox / chaperone properties provide precision and versatility to PDI(s) in redox signaling, making them promising therapeutic targets. Our group investigates the importance of redox processes as central determinants of vascular remodeling, however their mechanisms are unclear. We provided evidence that superoxide dismutase under-activity supports constrictive remodeling in the injury/repair model. Recently we showed that extracellular PDIA1 supports expansive caliber remodeling in conduit vessels during injury repair, via matrix/ cytoskeletal organization and beta1 integrin signaling. PDI inhibitors can address, at distinct levels, PDI effects in thrombosis, cancer and neurodegeneration.

Francisco R.M. Laurindo graduated in Medicine by the University of São Paulo, School of Medicine, São Paulo, Brazil, in 1978 and completed his residence training in Internal Medicine and Cardiology at the Heart Institute (Incor), University of São Paulo School of Medicine. After a period (1982-1984) as associate physician at the same institution, he underwent research training in Physiology and Pharmacology at the Uniformed Services University of the Health Sciences, in Bethesda, Maryland (under Dr. Robert Goldstein, 1984-1987). Back to the Heart Institute, University of São Paulo School of Medicine, he started an investigative research track focused on mechanisms of redox signaling processes in the vascular system. He obtained his PhD in 1992, supervised by Dr. Protásio da Luz. Thereafter, he conducted several research projects through independent financial support from research agencies. In 2001, he started, together with Dr. Protásio da Luz, the Vascular Biology Laboratory at the Heart Institute (Incor), University of São Paulo, School of Medicine and became its Director in 2008, until presently. His major research interests have focused on understanding mechanisms and regulatory processes underlying the production of oxidant species in vascular cells and tissues and their physiological implications for vessel remodeling in disease. The most important contributions of his group have been the original description of shear stress-dependent generation of superoxide radical from the endothelium, the multi-level characterization of redox response to vascular injury and, particularly, the original discovery that the endoplasmic reticulum chaperone protein disulfide isomerase interacts functionally and physically with oxidant-generating NADPH oxidase complexes. This finding had relevant implications regarding the role of endoplasmic reticulum pathophysiology on NADPH oxidase function and how redox processes regulate cell migration and vascular remodeling. More recently, Dr. Laurindo's group showed an important role for extracellular PDI in arterial remodeling after injury. Dr. Laurindo has authored or co-authored over 140 publications in peer-reviewed journals, cited >3,400 times (h-index=33). He supervised 18 PhD students and 17 post-doctoral fellows, in addition to several undergraduate trainees. From 2008-14, he was the vice-coordinator of the National Institute for Science and Technology of Redox Processes in Biomedicine (CNPq/Fapesp) and since 2013 the Vice-coordinator and principal investigator of Cepid-Fapesp Redoxoma. He is a member of the Brazilian Academy of Sciences since 2012 and a member of its Board of Directors since 2016, a member of Fapesp Study Committee in Health Sciences from 2008-2016 and Fapesp Adjunct Study Committee in Life Sciences since 2016, a member of Capes Federal Agency Committee (Medicine) from 1998-2017. He served as Council Member of the Society for Free Radical Biology and Medicine from 2010-2014 and belongs to the Editorial Board of Free Radical Biology and Medicine since 2008 and of Clinical Science since 2012. He has been an ad-hoc consultant for >30 publications and research agencies from Brazil and abroad. He was elected as vice-chair (2014) and chair (2016) of the Gordon Research Conference on Nox Family NADPH Oxidases.

Cardio-protection through the Modulation of Histone acetylation: Impact on mTOR regulation in two models of cardiac disease.

Gillette T G[1], [1]Cardiology University of Texas-Southwestern Medical Center.

Alteration of chromatin structure, by the post-translational modification of histones, plays a vital role in governing the activation and repression of gene transcription. The homeostasis of histone modification is a fundamental regulator of the transcriptional reprogramming that occurs in the setting of disease-related stress. Indeed, histone modification has been shown to be a key regulator of pathological growth and remodeling of the heart. Our studies have uncovered a link between histone modification and mTOR activation in cardiac myocytes in two distinct models of cardiac disease. The availability of small molecule inhibitors, to impact the architecture of chromatin and alter the transcriptional response to stress, suggests therapeutic potential in targeting pathological cardiac remodeling.

Thomas G. Gillette received his PhD from New York University Sackler Institute, where he did his thesis work focusing on DNA replication. From that time on his research efforts have been primarily centered on the role of cellular responses to stress, either environmental, physical or metabolic. He moved from New York down to Dallas, Texas to University of Texas Southwestern Medical Center in 1998 and started his post-doctoral work with Errol Friedberg. His work uncovered a non-canonical role for the 26S proteasome in response to UV irradiation. In 2004 he was appointed as faculty in Physiology at University of Texas Southwestern Medical Center, and continued his work on both canonical and non-canonical roles of the ubiquitin proteasome pathway (UPP) in cell stress responses. In 2009 he transferred to the department of Internal Medicine at University of Texas Southwestern Medical Center and was promoted to Associate Professor in 2016. His recent research directions are on understanding the molecular mechanisms of heart disease, focused primarily on the cellular responses to cardiac stress.

Genetic, biochemical, and physiological approach to understanding the function of Herp and Derlin family proteins in ER-associated degradation machinery.

Kokame K[1], Eura Y[1], [1]Department of Molecular Pathogenesis National Cerebral and Cardiovascular Center.

Endoplasmic reticulum (ER)-associated protein degradation (ERAD) is a cellular quality control system for the elimination of misfolded proteins in the ER. The ERAD machinery consists of multiple proteins that function cooperatively on the ER membrane. To explore the physiological requirement for ERAD factors, we generated Derlin-1–, Derlin-3–, and Herp-deficient mice. Complete loss of Derlin-1 caused embryonic lethality. Derlin-3– and Herp-deficient mice were born alive with the expected Mendelian frequency. In the Derlin-3– and Herp-deficient mouse organs, however, the expression levels of ERAD factors were affected under both normal and ER stress conditions; specific effects differed among the organs.Therefore, Herp and Derlin-3 may function in the regulation of the ERAD complex. Biochemical analysis using these mice demonstrated multiple ERAD machinery complexes in liver. Complex-1 appeared in two forms depending on the state of the ER: Complex-1a, under normal conditions, contained Herp, Derlin-2, and HRD1; and complex-1b, under ER stress, also contained Derlin-3. Complex-2, specifically observed under ER stress, contained Derlin-1, Derlin-2, VIMP, and p97. Derlin-3 was required for the ER stress-dependent changes in ERAD complex formation and the loss of Derlin-3 led to the decreased ERAD activity. Considering the highly-restricted expression of Derlin-3, these findings suggest that Derlin-3 may function as a sensing regulator of the stress condition of the ER.

Koichi Kokame. Department of Molecular Pathogenesis, National Cerebral and Cardiovascular Center, Osaka, Japan. He was born in 1967 in Hiroshima (Japan), grew up in Hiroshima, Fukuoka, and Kyoto, and now lives in Osaka. He entered Faculty of Science, Kyoto University in 1985. Then, he went on to Department of Biophysics, Graduate School of Science, Kyoto University in 1989, and undertook a basic study on the light signal transduction in photoreceptor cells. He finished a doctorate of Science at Kyoto University in 1994. His thesis was based on a paper entitled “Lipid modification at the N terminus of photoreceptor G-protein alpha-subunit” (Nature 359, 749). In 1994, he joined Department of Molecular Pathogenesis, National Cerebral and Cardiovascular Center in Osaka as a staff scientist, and shifted research subjects from photoreceptor cells to hemostasis and thrombosis. One of his current research subjects is endoplasmic reticulum (ER)-associated protein degradation (ERAD). In 1996, he published a paper entitled “Homocysteine-respondent genes in vascular endothelial cells identified by differential display analysis. GRP78/BiP and novel genes” (J Biol Chem 271, 29659). One of the novel genes was HERPUD1, which encodes an ER-membrane protein Herp. Herp expression is strongly induced by ER stress, and Herp is one of the key proteins in ERAD. The other subjects of his research are plasma proteins. In 2002, he published a paper entitled “Mutations and common polymorphisms in ADAMTS13 gene responsible for von Willebrand factor-cleaving protease activity” (PNAS 99, 11902). The functional deficiency of ADAMTS13 (metalloprotease) leads to thrombotic thrombocytopenic purpura (TTP), and that of von Willebrand factor (VWF, a substrate of ADAMTS13) leads to von Willebrand disease. His main impact has been made in the applicability of ADAMTS13 activity assay, which is widely used for the diagnosis of TTP.He became a principal investigator (Director, Department of Molecular Pathogenesis) in 2015, and the laboratory members are studying ERAD, ADAMTS13, VWF, and so on.

Andrew Quest got his PhD from the Swiss Federal Institute of Technolgy (ETH) Zuerich, Switzerland and went on to train as a post-doctoral fellow at the University of Washington, Seatlle, WA, USA and then at Duke University, Durham, NC, USA. During this training period he developed a keen interest in mechanisms of protein compartmentalization and particularly, in the case of Protein Kinase C, how interactions with lipids define protein function. He moved on to a position as assistant professor at the University of Lausanne in Switzerland where his focus changed to the membrane-associated scaffolding protein Caveolin-1 (CAV1), and his group then showed that it functions as a tumor suppressor in colon cancer cells. In 1999, he took up a position as Associate Professor in the Department of Cell and Molecular Biology at the Faculty of Medicine, University of Chile. In 2005, he became Full Professor at the same institution. In Chile, he continued working on the role of CAV1 as a tumor suppressor and discovered that the protein modulates the Wnt-signalling pathway and controls b-catenin/Tcf-Lef-dependent transcription of the cancer-related genes survivin and cyclo-oxygenase-2, but only does so in cancer cells that still express E-cadherin. His group then turned to studying what CAV1 does in the absence of E-cadherin and showed that the protein promotes metastasis and does so by activating a novel signaling axis linking Rab5 to Rac1 activation. Thus, CAV1 plays a dual role in cancer by promoting metastasis in the absence of E-cadherin, but acting as a tumor suppressor in the presence of E-cadherin. In more recent years, his group has turned to the study of mechanisms by which Helicobacter pylori infection promotes the development of gastric cancer, the leading cause of cancer deaths in Chile, as well as the role of chronic inflammation in promoting the development of cancer. In the course of the latter studies, CAV1 has emerged as a significant player in modulating the unfolded protein response (UPR) of the ER, as well as ER-mitochondria communication. Andrew Quest is currently director of the Center for Molecular Studies of the Cell (CEMC) and Principle Investigator of the Advanced Center for Chronic Diseases (ACCDiS).

Symposium

Capillary electrophoresis for Biochemical and Molecular Biological applications
Chair: Norberto Guzman

An Immuno-Analytical Separation Instrument for the Determination of Bioactive Peptides in Biosamples – Implication in Inflammation-Associated Diseases.

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There is an abundance of bioactive peptides contained in a wide range of food sources, which are generated by fermentation, enzymatic cleavage, chemical hydrolysis, or gastrointestinal digestion processes from food proteins. Many of these peptides have beneficial properties to humans while others may adversely affect human health. In the case of milk and derived dairy products, which are considered important constituents of a balanced diet, they are also the source of bioactive peptides capable of acting on particular cellular receptors. Among these peptides, beta-casomorphin 7 (BCM7), derived during hydrolysis of milk A1 variant beta-casein, contains a histidine residue at position 67 and has been of significant interest. It has been suggested that BCM7 contribute to an increase risk of certain inflammation-associated non-communicable diseases, including schizophrenia, autism, cardiovascular diseases, and type-1 diabetes. BCM7 and related peptides are known as opioid peptides because they behave as opioid receptor ligands. The role of beta-casomorphins remains controversial and more research with improved diagnostic techniques is needed to unravel the mechanism and to study the physiological functions of beta-casomorphins. In this presentation, I will discuss the determination of opioid peptides in biofluids using a miniaturized immuno-analytical portable instrument. The principle by which this instrument operates is based on a two-dimensional technology known as immunoaffinity capillary electrophoresis (IACE). IACE combines the use of antibodies, or other affinity ligands, as highly selective capture agents with the superior resolving power of capillary electrophoresis and capable of improved sensitivity, specificity and throughput.

Dr. Norberto Guzman received a B.Sc. degree in biochemistry (clinical biochemistry) from a Joint Undergraduate Program of the University of Concepcion and the University of Chile, Santiago, Chile; a M.Sc. degree in biochemistry (cell and molecular biology) from the Medical College of Georgia, Augusta, Georgia, U.S.A.; and a Ph.D. degree in biochemistry (protein biochemistry) from a Joint Graduate Program of the University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School (formerly Rutgers Medical School) and Rutgers, The State University of New Jersey, New Brunswick, New Jersey, U.S.A. Dr. Guzman has worked for the last 30 years in academic medical institutions, diagnostic and pharmaceutical companies, including Mount Sinai School of Medicine, Roche Diagnostic Systems, Hoffman-La Roche, and Johnson & Johnson. He also has worked in a collagen food-nutraceutical industry. Dr. Guzman is the author or co-author of more than 130 scientific publications, including manuscripts, patents and book chapters. He has delivered over 300 oral presentations in Europe, the Americas, the Far East, and Australia. According to Google Scholar Citations, Dr. Guzman's publications have been cited more than 5000 times, having an h-index of 37, and an i10 index of 67. One publication alone has reached 1300 citations. Seven figures of his publications have appeared on the front cover of prestigious scientific journals and books. One presentation at Google Headquarters in New York City has been viewed more than 1700 times (https://www.youtube.com/watch?v=1QnTrcYWk-o). He is the editor of two widely referenced books on the subject of capillary electrophoresis and collagen prolyl hydroxylase. Dr. Guzman holds numerous worldwide patents on capillary electrophoresis and microchip technology and his accomplishments have been recognized by being the recipient of many national and international awards in science and technology innovation. Dr. Guzman is a member of several international scientific organizations. He serves on the editorial board of Electrophoresis (European journal), and the Journal of Liquid Chromatography and Related Technologies (American journal). Dr. Guzman is the founding editor of the Journal of Capillary Electrophoresis and Microchip Technology and one of the pioneers in this field. He is also the founder of the international symposia

series known as LACE (Latin-American Capillary Electrophoresis). Dr. Guzman is currently Chief Scientific Officer at Princeton Biochemicals Inc., Princeton, New Jersey, U.S.A. Dr. Guzman's expertise is primarily in biomedicine and biotechnology with emphasis in protein biochemistry and immunochemistry. At present, his main research interest is in the understanding of the function of newly-formed and/or post-translational-modified proteins in inflammatory processes, and the finding of therapeutic agents, such as natural and synthetic proteins/peptides, aimed to alleviate chronic inflammatory diseases. For several years, Dr. Guzman has developed immunoaffinity-analytical separation instrumentation and methodologies for the quantification, identification and characterization of proteins and peptides of relevance to the clinical laboratory, pharmaceutical industry and food-nutraceutical industry (e.g., erythropoietin, antibodies, and collagen). He also has used multiple cross-linking methods to generate scaffold of collagen with other natural or synthetic polymers to generate collagen-based biomaterials for use in tissue engineering applications or microencapsulation technology. References: 1. Electrophoresis 32(13): 1565-1578 (2011), Guzman NA, Phillips TM. 2. Atlas of Science – Another View of Science March 21 (2016), Guzman NA, Guzman DE. 3. Journal of Chromatography B, 1021: 14-29 (2016), Guzman NA, Guzman DE. 4. U.S. Patent Numbers 6,406,604; 7,153,407; 7,329,388; 7,736,480; 7,811,436; 8,007,724; 8,007,725; 8,182,746; 8,268,247; 8,703,061; 8,865,075; 9,146,234; 9,482,602 (2002-2016), Guzman NA; European Patent Number EP 1706735 (2017), Guzman NA.

Comprehensive Characterization of N-Linked Carbohydrates by CE-LIF AND CESI-MS.

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There is a growing interest in the biopharmaceutical industry and the biomedical field for rapid and high-resolution N-glycosylation analysis of glycoproteins. The recently introduced fast glycan labeling and analysis approach for N-linked carbohydrate profiling of glycoproteins offers rapid and high-resolution separation of the target carbohydrate molecules. Glycan release, fluorophore labeling and clean-up parameters were all optimized resulting in 60 min sample preparation time using a novel magnetic bead mediated process that assures excellent yield, high reproducibility and easy automation. Optimization included rapid endoglycosidase digestion and fluorophore labeling reaction conditions (time and temperature), also addressing efficient clean-up and rapid capillary electrophoresis-laser-induced fluorescence (CE-LIF) separation-detection. The process can be readily implemented to automated liquid handling platforms, not necessitating any centrifugation and vacuum centrifugation steps, otherwise necessary for most glycan sample preparation methods. Albeit, CE separation provides high resolution and migration time-based identification of glycans using the built in glucose unit (GU) database, coupling with MS detection offers additional structural verification. Integration of CE and electrospray ionization (ESI) into a single dynamic process (CESI) provides the capability of performing CE separation and MS ionization with ultra-low flow rates, resulting in reduced ion suppression and improved sensitivity. Utilizing novel MS-compatible buffer systems, our results revealed that in negative ionization mode with reversed CE polarity (and consequently negative EOF) and low forward pressure resulted in excellent separation performance to obtain comprehensive glycan analysis data.

Dr. Jeff Chapman currently holds the position of Senior Director, CE Business, at SCIEX, a Danaher Company. Jeff and his team are responsible for the development, manufacturer, sales and support of electrodriven microscale separations technology at SCIEX. Jeff holds B.Sc. and Ph.D. degrees in Biochemistry, Immunology and Neuroimmunology from the University of Saskatchewan and the University of Calgary. He joined Beckman Instruments in 1989 in Mississauga, where he was responsible for introducing Capillary Electrophoresis (CE) technology to Scientists across Canada. In 1995, he moved to Southern California, where he took on worldwide responsibility for CE. Beginning in 1998, Jeff moved into the development side of Beckman Coulter and has held the positions of Product Manager, Strategic Marketing Manager, Director of Scientific Alliances and Director of Global Marketing. Jeff transitioned the CE business over to SCIEX in 2013, integrating CE with nano-LC and micro-LC to form one of the largest microscale separations operations in the World. Dr. Chapman has more than 30 years of experience with CE technology over his career, has served on myriad scientific committees and boards on this topic, and is cited often as an industry expert in this field. He is also serving currently as a member of the Strategic Planning Committee for MicroScale Bioseparations.

Advances in the Separation of Co- and Post-translational Modifications using Capillary Electrophoresis – Mass Spectrometry.

Lindner H[1], Sarg B[1], Faserl K[1], [1]Division of Clinical Biochemistry Innsbruck Medical University.

Comprehensive characterization of protein co-translational (CTMs) modifications and post-translational modifications (PTMs) still challenges the modern analytical platforms due to their inherent nature of low abundance and low stoichiometry. Traditionally, liquid chromatography coupled to mass spectrometry (LC-MS) is the analytical technique of choice for the analysis and quantification of modified proteins and peptides. However, each CTM and PTM shows different characteristics and, therefore, often requires different approaches to analyze. Alternative analytical platforms such as capillary electrophoresis coupled to mass spectrometry (CE-MS) can be extremely informative and useful due to method inherent advantages, e.g. CE allows separations based on the mass-to- charge ratio of peptides and does not utilize their hydrophobic nature as a separation principle. Method-inherent advantages of CE-MS are highly efficient separations, low flow rates leading to reduced ion suppression, and greater sensitivity, among others. This work describes the suitability of low-flow CE-MS for the identification of different co- and post-translational modifications, e.g. acetylation, glycosylation, phosphorylation and methylation in medium complex protein samples. Further, the CE-MS platform has been used to detect challenging modifications including deamidation products in crude protein fractions. The method can also be used to detect citrullination in intact and digested proteins and in combination with tandem MS and electron transfer dissociation (ETD) fragmentation, locate the presence of deiminated Arginine. Finally, its ability to separate and quantify individual positional isomers of isobaric mono-phosphorylated peptides obtained in the course of a kinase activity study will be demonstrated. Based on the results obtained CE-MS can be considered a complementary technique to conventional LC-MS and an alternative approach for CTM and PTM analysis. Principles, advantages and disadvantages of both techniques are discussed in detail.

Dr. Herbert Lindner began his undergraduate education in the field of chemistry at the University of Innsbruck, Austria. In 1982, while employed as a Contract Assistant at the Institute of Organic and Pharmaceutical Chemistry, he obtained his Ph.D. Later in the same year, he relocated within the University to a position of Contract Assistant at the Department of Medical Chemistry and Biochemistry and, subsequently, was promoted to the position of University Assistant in 1984. In 1992, he received the award of Habilitation and “venia legendi” for Biochemistry. He was promoted to the position of Assistant Professor in 1994, and then to Associate Professor three years later. In 2007 Dr. Lindner was appointed Head of the Protein Micro-Analysis Facility at Innsbruck Medical University. He is currently the Vice Director of the Division of Clinical Biochemistry at Innsbruck Medical University.

Dr. Lindner is the author and co-author of more than 130 scientific publications, including manuscripts, reviews and book chapters. His publication citations are reaching 3400 times, having an h-index of 33, and an i10 index of 83, according to Google Scholar Citations. He has delivered over 250 oral presentations in Europe, the Americas and Asia. He presented several webinars about the use and principles of low-flow sheathless capillary electrophoresis-electrospray ionization-mass spectrometry in proteomics research.

Dr. Lindner’s main teaching responsibilities are in chemistry, analytical-bioanalytical chemistry, biochemistry, mass spectrometry, advanced topics in clinical biochemistry and bioanalysis. He also teaches theory and practical training to medical students on mass spectrometry, high-performance liquid chromatography, high-performance capillary electrophoresis, and the sequencing of amino acids in a peptide by the Edman degradation method.

Dr. Lindner’s laboratory focuses on the development of high-resolution methods for the separation and identification of post-translationally modified proteins in order to investigate their biological significance. A set of separation methods based on capillary electrophoresis (CE), reversed-phase chromatography, hydrophilic interaction liquid

chromatography (HILIC) and mass spectrometry (MS) was introduced in his laboratory. In addition and as a result of a continuous development program over many years, he offers a wide range of analytical methods and services to support the work of other research scientists at the University and Industry (https://www.i-med.ac.at/iftz/zentrale_gruppen/proteinfacility/). The analytical tools developed and routinely applied in his laboratory led to numerous publications and successful national and international collaborations.

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A Miniaturized Solid-Phase Extraction Method Coupled On-Line to Capillary Electrophoresis and Mass Spectrometry for High-Sensitivity Analysis of Biomolecules.

Benavente F[1], Pont L[1], Peró-Gascon, R[1], Giménez E[1], Barbosa J[1], Sanz-Nebot V[1], [1]Department of Chemical Engineering and Analytical Chemistry, Nutrition and Food Safety Research Institute, Faculty of Chemistry, University of Barcelona.
Capillary electrophoresis mass spectrometry (CE-MS) has been widely used for the analysis of a great variety of biomolecules and it is regarded as an excellent complement to liquid chromatography mass spectrometry (LC-MS). However, one of the major drawbacks of CE-MS, and other microscale separation techniques, are the poor concentration limits of detection. The high-resolution power of CE and the excellent selectivity of MS permit precise separation and characterization of the target compounds, but the limited loading capacity needed for optimal separation in CE hinders the analysis of diluted samples. Several strategies have been developed to improve CE-MS sensitivity. In this presentation, I will describe the CE-MS instrumental set-ups that currently provide the best performance in terms of reproducibility and sensitivity. Then, I will explain in detail how to further decrease the limits of detection using a miniaturized solid-phase extraction method coupled on-line to capillary electrophoresis and mass spectrometry with different affinity sorbents (C18, immobilized metals, antibodies, etc.) for clean-up and preconcentration of low abundant components in biological samples, from small peptides or metabolites to high molecular mass proteins, with emphasis on biomedical applications.

Dr. Fernando Benavente received his M.Sc. in Chemistry and Ph.D. in Analytical Chemistry in 1998 and 2003 at the University of Barcelona (UB, Barcelona, Spain). He is currently an Associate Professor in the Department of Chemical Engineering and Analytical Chemistry of the UB, a member of the Nutrition and Food Safety Research Institute of the University of Barcelona (INSA-UB) and one of the leaders of the group of Bioanalysis. Dr. Benavente joined this group in 1997, and he has also conducted doctoral and postdoctoral research at The RW Johnson Pharmaceutical Research Institute (Raritan, New Jersey, USA), The National University of Rosario (Rosario, Argentina) and the University of Leiden (Leiden, The Netherlands). His research is focused on the development and application of high performance separation techniques coupled to mass spectrometry to solve complex analytical problems related to biomedicine, pharmaceuticals, food industry and forensic sciences. Dr. Benavente is especially interested in the separation, sensitivity enhancement, characterization, and reproducibility of the analysis of peptides, proteins, glycoproteins, oligomeric proteins, metabolites and bioactive compounds in biological samples, biopharmaceuticals and food using LC-MS, CE-MS and related techniques. He is an expert in the development and application of on-line solid phase extraction capillary electrophoresis mass spectrometry (on-line SPE-CE-MS). His contributions include more than 80 international peer-reviewed publications, several book chapters and more than 100 presentations at national and international conferences. In 2014 the journal The Analytical Scientist included him in the ‘top 40 under 40’ of a worldwide selection of important analytical scientists.

Researchgate: www.researchgate.net/profile/Fernando_Benavente
Research Group Website: www.ub.edu/bioanalisi

Symposium

An approach to the Infectious Pancreatic Necrosis Virus: from the virus to the host
Chair: Andrea Rivas

Infectious Pancreatic Necrosis Virus Morphogenesis.

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Infectious Pancreatic Necrosis Virus (IPNV) is widespread in all salmon farming around the world, producing economic losses due to morbidity and mortality. Their genome consists of two segments of double-stranded RNA, belonging to the *Birnaviridae* family. Is a non-enveloped icosahedral virus and is formed by four proteins. The VP1 protein correspond to the viral polymerase, encoded by the major RNA segment, also acts as VPg covalently linked to the 5 ´end of both RNA segments. The VP2 protein, which forms the viral capsid, is synthesized as part of a polyprotein along with VP3 and VP4, all encoded by the minor segment of RNA. VP4 is the viral protease which with VP3 forms the viral core. We observed by southern blot that the IPNV replication occurs through a negative strand intermediary which is used as template of the positive strand. In experiments where both nucleic acid and proteins were labeled with radioactivity, it was observed that once the double stranded RNA replication occur apparently genome acts as a nucleation center for the formation of a provirus. With different proteases inhibitors we demonstrates that the provirus is proteolytically processed by the viral protease to become an infectious and mature virion. These events of the replicative cycle will be discussed in the symposium.

Dr. Ana María Sandino obtained her bachelor degree in biochemistry at the Universidad de Chile in 1984 and her PhD in Science in 1990 at the same University. In 1990 she became Assistant Professor of the Institute of Nutrition and Food Technology (INTA) at the Universidad of Chile and created her line of research in the study, both basic and applied, about viruses that infect salmon. In 1995, she became Associate Professor and joined the Laboratory of Virology of the Department of Biology of the Faculty of Chemistry and Biology at the Universidad de Santiago de Chile. Since, 2004 she is a Full Professor, and her research has been. focused to the study of the molecular mechanisms of the pathogenesis of the IPN virus.. In addition, in 1997 Dr. Sandino co-founded the biotechnology company Diagnotec S.A, where she was Scientific Director . In 2011 he was co-founder of ActivaQ, and until now the Scientific Director of this biotech company.

Translation initiation of Infectious Pancreatic Necrosis Virus mRNAs.

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Infectious pancreatic necrosis (IPN) is a highly prevalent salmonid disease that affects Chilean salmon farming. IPNV, the causative virus, is a non-enveloped icosahedral virus, with a genome composed of two segments of double-stranded RNA (ds) RNA (RNA-A and RNA-B). Both segments are uncapped and nonpolyadenylated. Instead of a cap, the 5-ends of the two strands of the viral segments are linked covalently to a viral genome-linked protein (VPg). The structural characteristics of viral mRNA have to be relevant during its translation.
The recruitment of the 40S subunit of the ribosome to the mRNAs is gathered by eukaryotic translation factors (eIFs), following different mechanisms. In the canonical mechanism, the recruitment of the translation initiation machinery occurs on the cap presented in the 5-end of the mRNAs. In the IRES-dependent mechanism, the machinery is recruited to an internal ribosome entry site (IRES) in the 5 untranslated region (5'UTR) of the mRNA. Besides, there is another mechanism displayed by some viral mRNAs whose 5'-extreme is attached covalently to a viral protein linked to the genome (Vpg), which could recruit the translation machinery.
IPNV infection inhibits the cellular translation, specifically cap-dependent translation, by activation of PKR and PERK kinases followed by phosphorylation of the eukaryotic translation factor-2 (eIF2). However, this inhibition does not affect viral translation, and protein accumulates into the cell. This evidence indicates that IPNV mRNAs exert an alternative mechanism to the Cap-dependent to translate their proteins. These mechanisms will be discussed in this symposium.

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Dr. Andrea Rivas Aravena was graduated as Biochemist at Universidad de Santiago de Chile. In 2009 she got her Ph.D. in Sciences, mention in Molecular and Cellular Biology at the Pontificia Universidad Católica de Chile, working on the regulation of translation initiation of HIV-1 full-length mRNA, under supervision of Dr. Marcelo López. The same year, she started a postdoc on the study of the viral replicative cycle of fish viruses as Infectious Salmon Anemia Virus (ISAV), salmonid alphavirus (SAV), and Infectious Pancreatic Necrosis Virus (IPNV) and their interaction with the host mediated by the immune system. Since 2015 she is a researcher at the Comisión Chilena de Energía Nuclear. Her research group is divided into two areas that involve the study of the regulation of the initiation translation of IPNV, and the interaction of ionizing radiation with viruses and other biology system.

Immune response of salmonids against Infectious Pancreatic Necrosis Virus.

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IPNV is the agent of a well-characterized acute disease that produces systemic infection and high mortality in farmed salmonid species. In order to understand anti-IPNV immune response of salmonids, we have studied several conditions of infections and characterized the response mostly at the transcription level. In one of these studies, we have analyzed IPN-susceptible and resistant families of Atlantic salmon and studied expression of the immune-related genes in kidneys associated with these phenotypes at day 1 and 5 post-infection using microarrays. Transcripts involved in antigen presentation (HSP-70, HSP-90, MHC-I), TH1 response (IL-12, IFN-g, CRFB6), immunosuppression (IL-10, TGF-b1) and leukocyte activation and migration (CCL-19, CD18) showed a differential expression pattern between both phenotypes. Interestingly, in susceptible families, the expressions are high on day 1 and dropped to basal values on day 5 post-infection whilst in resistant families, levels rise moderately on day 1 and remained high or increased at day 5. This suggests that a limited but sustained immune response is associated with resistant fish phenotype to IPNV challenge while a highly inflammatory but short response is associated with susceptibility. Another characteristic of IPNV is its ability to produce persistent infection. Analysis of the cytokine levels during acute and persistent infection showed a distinct profiles of expression in kidney and spleen. Interestingly, the balance between pro-inflammatory Th1 type cytokines and the regulatory cytokines can explain the high percentage of survival and the resolution of the inflammatory response in the IPNV-infected fish but also the establishment of viral persistence.

FONDECYT 1161015.

Dr. Mónica Imarai obtained her PhD at the Pontificia Universidad Católica de Chile, and did her PhD thesis at the Albert Einstein College of Medicine, NY, under the supervision of Dr. Stanley G. Nathenson investigating the diversity of T cell receptors specific for the VSV antigenic peptide (N52-59) bound by the H-2Kb class I molecule. Since 1993, she has worked at the Universidad de Santiago de Chile where she created the Laboratory of Immunology and actually led a research group in fish immunology. In this field, she is contributing to understand adaptive immunity of the trout and salmon, developing new tools and assays to analyse salmon immune responses to infections, vaccines or functional diets but also to understand the mechanisms of immune response.

Deciphering of clonal complexity of b cell response in trout using deep sequencing.

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Fish infection or vaccination induces the production of antigen-specific antibodies by B lymphocytes. These B cells are recruited based on the specificity of their surface antibody, among the vast diversity of receptors (more than 10^{11}) produced through the random and imprecise genomic rearrangement of V, D and J genes during lymphocyte differentiation. In fish, the monitoring of B cell response to infections or vaccines has been mainly performed by serological and molecular techniques that provide a limited insight into the complexity of humoral adaptive immune response. We have developed a deep sequencing based approach to compare the clonal structure of the systemic or mucosal rainbow trout B cell response induced by pathogens and vaccines. In this approach, unique barcode labels are incorporated on each starting cDNA molecule before amplification, allowing the correction of PCR/sequencing errors by generating consensus sequence and a safer quantification of sequence relative abundance. We characterized the clonal complexity of the IgM and IgT repertoire, revealing different structures before and after immunization. Our data will be useful to model the development of the Ig landscape, and to understand the mechanisms of B-cell memory after infection by pathogens or vaccination in fish.

Dr. Susana Magadán obtained her bachelor degree in Biochemistry and Molecular Biology at Universidad Autónoma de Madrid (Spain) and her PhD at the Universidad de Vigo (Spain) working on the production of fully human monoclonal antibodies directed to human leukemia cells using transgenic mice. After working as Associate Professor at the “Instituto Superior de Saúde do Alto Ave” (Portugal), she returned to Spain to join the Instituto Español de Oceanografía (2009) where she was employed as Associate Researcher to develop different studies in fish comparative Immunology, from a genomic and functional stand points. Since then, she has been working in different centers, at the Institute National de la Recherche Agronomique in France (2012-2015), at Center of Evolutionary and Theoretical Immunology, University of New Mexico, USA (2016) and currently, at the Universidad de Vigo (Spain), to implement new “OMICs” tools to decipher the adaptive immune response in fishes against pathogens affecting to Aquaculture industry, like VHS and IPN virus. She will present this work in the conference under the title “Deciphering the complexity of antiviral B cell response in trout by deep sequencing”.

Oral Sessions 1

Biomedicine and Gene Expression

Serum levels of Interleukin-6 are linked to the severity of the disease caused by Andes Virus.

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Andes virus (ANDV) is the etiological agent of hantavirus cardiopulmonary syndrome in Chile. In this study, we evaluated the profile of the pro-inflammatory cytokines IL-1β, IL-12p70, IL-21, TNF-α, IFN-γ, IL-10 and IL-6 in serum samples of ANDV-infected patients at the time of hospitalization. The mean levels of circulating cytokines were determined by a Bead-Based Multiplex assay coupled with Luminex detection technology, in order to compare 43 serum samples of healthy controls and 43 samples of ANDV-infected patients that had been categorized according to the severity of disease.When compared to the controls, no significant differences in IL-1b concentration were observed in ANDV-infected patients (p=0.9672), whereas levels of IL-12p70 and IL-21 were significantly lower in infected cases (p=<0.0001). Significantly elevated levels of TNF-a, IFN-γ, IL-10, and IL-6 were detected in ANDV-infected individuals (p=<0.0001, 0.0036, <0.0001, <0.0001, respectively). Notably, IL-6 levels were significantly higher (40-fold) in the 22 patients with severe symptoms compared to the 21 individuals with mild symptoms (p=<0.0001). Using multivariate regression models, we show that IL-6 levels has a crude OR of 14.4 (CI: 3.3-63.1). In conclusion, the serum level of IL-6 is a significant predictor of the severity of the clinical outcome of ANDV-induced disease.

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Rational design and biological activity of synthetic compounds as blockers of potassium channels TASK.

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Potassium (K+) channels are part of one of the most abundant family of transmembrane proteins. K+ channels allow the selective transfer of K+ ions inside or outside of the cell. Within this family we find K_2P channels, with four transmembrane segments and two pore domains per subunit to form a functional dimer. These channels are expressed in the central nervous system, cardiovascular system, genitourinary system and gastrointestinal system. They are related with several pathologies in humans. Thus, members of this family have emerged as molecular candidates for the action of pharmacological agents. The K_2P subfamily channel TASK has been verified as an oncogene. Its overexpression accelerates human tumor formation such as in ovarian and breast carcinomas. These channels are blocked by a variety of compounds and molecules, such as A18999, bupivacaine, Zn^{2+}, doxapram, loratadine, mevastatin, mibefradil, and octoclothepin and also by congeneric series such as THPP-derived compounds and bis-amide derived compounds. In this work we synthesize new compounds as selectivity blocker of TASK channels. The compounds are based in rational design and bioinformatics studied. The base of compounds is bis-amide substituted, then, the compounds were tested as blockers in electrophysiological assays. The results were very promising, several compounds with activities at nanomolar level. To correlate the results the compounds were tested on cancer cell lines.

Activation of the Calcium-Sensing Receptor (CaSR) induces LS14 preadipocyte inflammation through mitochondrial dysfunction.

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Inflammation is a major contributor to adipose tissue (AT) dysfunction, which in turn is responsible for obesity-associated pathologies. Recent investigation has shown that the Calcium-Sensing Receptor (CaSR) participates in AT dysfunction by promoting pro-inflammatory cytokine secretion in adipocytes and preadipocytes. These cell types are key whole-body metabolic regulators; however, the extent of mitochondrial contribution to their pathogenesis remains unknown. Using cinacalcet (2 µM), a CaSR-specific allosteric activator, we studied whether CaSR activation (20 h) affects mitochondrial function in the human liposarcoma preadipose cell line LS14. mRNA levels were assessed via qPCR. Mitochondrial morphology and endoplasmic reticulum (ER)-mitochondria proximity were assessed via confocal microscopy of samples stained with MitoTracker Orange (mitochondria) and anti-KDEL antibody (ER). Mitochondrial respiratory function was determined through oxygen consumption using a Clark electrode, and mitochondrial potential was assessed via flow cytometry using tetramethylrhodamine (TMR). As reported, exposure to cinacalcet increased the mRNA levels of pro-inflammatory cytokines in LS14 preadipocytes. This effect that was prevented by decreasing mitochondrial bioenergetics with CCCP (200 nM), whereas augmenting mitochondrial with methyl pyruvate (200 mM) exacerbated it. While ER-mitochondrial contacts appeared unaltered by cinacalcet treatment, the mitochondrial network underwent fragmentation. Concomitantly, both mitochondrial transmembrane potential and respiratory rate were diminished by 20–30% in these cells. These results indicate that mitochondrial function contributes to CaSR-mediated inflammation. However, after cinacalcet treatment mitochondria display decreased activity. Thus, we propose that CaSR activation promotes inflammation in LS14 preadipocytes through a mechanism that exacerbates mitochondrial bioenergetics, ultimately leading to organelle dysfunction when prolonged in time.

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Development and inhibitory mechanism of a neutralizing molecule against Andes virus *(Hantaviridae)*

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Hantaviruses belong to the order *Bunyavirales* and are harbored by rodents and small insectivors. In Chile and other South American countries, the Andes virus (ANDV) is endemic and is transmitted to humans mainly by *Oligoryzomys longicaudatus*, causing hantavirus pulmonary syndrome (HPS) with mortality rates ranging from 30 to 40%. To date, there are no effective HPS treatments approved by the Food and Drug Administration (FDA). ANDV has been reported to infect pulmonary vascular endothelial cells and the Gn and Gc viral glycoproteins mediate the virus cell entry processes including receptor binding, internalization, and subsequent virus-cell membrane fusion. This latter process is induced by the pH acidification in endosomes, which induces conformational changes in the Gc fusion glycoprotein. Here we aimed to develop molecules that bind to the ANDV glycoproteins and block the infection of cells. The developed molecules specifically recognize the ANDV glycoproteins in ELISA, indirect immunofluorescence assays and flow cytometry and are cross-reactive with other hantaviruses. One of these molecules has inhibitory activity against ANDV in *in vitro* infection assays. The binding site of this molecule to viral glycoproteins was identified by a peptide scan approach and its inhibitory activity involves the arrest of Gc in an intermediate of the membrane fusion process.

Proyecto FONDECYT 1140050 Proyecto FONDEF CA12|10367 Proyecto Basal PFB-16 Beca de Doctorado Nacional NM.

The relation of RCAN1 overexpression and mitochondrial dynamics in induced pluripotent stem cells (iPSCs) of Down Syndrome.

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

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

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

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

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Role of HERPUD1 and ERAD activation during maturation and mineralization of osteoblast in vitro

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Introduction: Bone is a highly dynamic tissue whose integrity is the result of a balance among bone synthesis by osteoblasts and bone resorption by osteoclasts. The high secretion capacity of mature osteoblast increase folding and quality control demand of Endoplasmic Reticulum (ER), inducing stress and activating of unfolded protein response (UPR) and ER-associated degradation (ERAD). UPR impairment have been related to bone physiopathology. However, in the osteoblast biology, ERAD, is fully unknown. HERPUD1 is an ER-membrane protein and the master organizer of ERAD multiprotein complex stability. Our aim is to stablish if HERPUD1 and the regulation of ERAD, play a role during the osteoblastogenesis.

Methods: Pre-osteoblast MC3T3-E1 cell line, was differentiated in presence and absence of HERPUD1 for 3, 7 and 14 days. Osteoblast differentiation was evaluated through increase in calcium deposits, alkaline phosphatase (ALP) activity and expression of differentiation genetic markers (r*unx2, osx, rankl* mRNAs). HERPUD1 levels was determined by qPCR and Western blot analysis. ERAD and proteasomal degradation activity was performed using GFPμ or CD3-YFP reporters, respectively.

Results: During osteoblast differentiation HERPUD1 increased since 12 h to 14 days. We confirm that ERAD and proteasomal degradation is activated during osteoblast maturation. The absence of HERPUD1 blocked the osteoblast mineralization, and reduced ALP activity and differentiation markers expression. Whereas, HERPUD1 overexpression was sufficient to activate the whole osteoblast differentiation program.

Conclusion: HERPUD1 and ERAD are important to activate the osteoblast maturation program and could be a new target to understand, diagnosis or treatment of bone pathologies.

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The small isoform of the HBx protein is sufficient to establish an active hepatitis B viral chromatin state

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About 350 million people around the world are chronically infected with the hepatitis B virus (HBV). Those people are at risk of developing hepatocellular carcinoma. The HBV genome is organized as a minichromosome in the infected hepatocyte. It encodes several viral proteins, including the protein X (HBx), a small non-structural protein that participates in multiple functions in infected cells. The HBx mRNA contains two internal in-frame AUG codons that could allow for alternative translation initiation, since the first AUG codon is in a poor context. In this work, we investigated whether the three isoforms, named as full (F), middle (M) and small (S) HBx, are expressed and are functional. By mutating the HBV genome, we found that the smaller HBx polypeptide is able to recapitulate the HBV transcription and replication levels of the wild type virus. Our results suggest that the smallest HBx polypeptide accomplish this by acting as an epigenetic regulator of the HBV minichromosome. Work supported by the grants from ANILLO ACT1119 (AL), Basal Project PFB16 (AL), FONDECYT 1120170 (AL), PCHA/Doctorado Nacional/2014-21140324 (FA), 2014-21140956 (SH).

CBP80/20-dependent translation initiation factor (CTIF) inhibits HIV-1 Gag synthesis by interfering with the Rev-CBP80 interaction

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Gag synthesis from the Human immunodeficiency virus type-1 (HIV-1) unspliced mRNA involves a non-canonical nuclear export pathway as well as cap-dependent and cap-independent mechanisms of translation initiation. The viral protein Rev is critical in ensuring proper Gag synthesis during viral replication by acting as the master post-transcriptional regulator of the unspliced mRNA. Here, we show that the nuclear cap-binding protein 80 (CBP80) promotes HIV-1 Gag synthesis by cooperating with Rev during nuclear export and translation. These functions of CBP80 were independent of the cap-binding function of the CBC but required the assembly of a specific mRNP in the nucleus. Interestingly, we identified the CBP80/20-dependent translation initiation factor (CTIF) as a negative regulator of Gag synthesis. This inhibitory function of CTIF was dependent on Rev and was exerted by its CBP80-binding domain suggesting that CTIF interferes with the Rev-CBP80 interaction. Together, these data reveals a new role of CBP80 in supporting Rev function during Gag synthesis and an unexpected role of CTIF as a restriction factor for HIV-1 gene expression.

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Oral Sessions 2

Animal and Plant Molecular Cell biology

Coagulation Factor Xa promotes melanoma metastasis

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A hypercoagulable state and complications from metastasis are the principal reasons for cancer-related mortality. Accumulate evidence suggest that the coagulation system plays a role in metastasis,and anticoagulants have been reported to be beneficial in prolonging cancer patient survival. However, the contribution of distinct proteins of coagulation cascade to cancer progression is still understood. The coagulation factor Xa (FXa), an effector protease of coagulation cascade, has a pivotal role among distinct cells types, such as: endothelial cells, fibroblast, mesangial cells and vascular smooth muscle cells. Nevertheless, the contribution of FXa to cancer cell biology and tumor progression is poorly described. Therefore, the principal aim of this study is to elucidate the contribution of FXa to cancer cell proliferation, migration, invasion (*in vitro)*, and the contribution to cancer cell metastasis (*in vivo*). The potential role of FXa upon metastasis was assessed using tail-vein injections of melanoma in the B16F10-C57BL/6 lung-metastasis model. The effect of FXa upon the biological properties of cancer cells was assessed *in vitro*, using boyden chamber (invasion assay), scratch assay (migration) and flow cytometry (cell cycle analysis). FXa significantly increased B16F10 melanoma lung metastasis in C57BL/6 mice, but FXa did not alter melanoma cell proliferation, migration or invasion capabilities in *in vitro* assays. Despite having no detectable effect upon the melanoma cells in culture, FXa can notably increase metastasis foci. FXa may promote metastasis by modulating the microenvironment of the metastatic niche.

Astrocytic Syndecan-4 binding to neuronal Thy-1 strengthens retraction and inhibition of neuronal processes triggered by Thy-1/αvβ3 integrin engagement.

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In a pro-inflammatory context, for instance following brain injury, reactive astrocytes undergo changes in surface protein expression that hinder axon regeneration.avb3 integrin is up-regulated in reactive astrocytes, interacts with neuronal Thy-1 to suppress neurite outgrowth and retract existing neuronal processes; however, blocking avb3 integrin only partially prevents these events, suggesting the participation of other molecules. We propose that the proteoglycan Syndecan-4, another Thy-1 receptor that is up regulated in reactive astrocytes, binds to Thy-1 strengthening integrin-mediated effects in neurites. Using neurons seeded over astrocytes and Syndecan-4 blocking/silencing, we tested the ability of Syndecan-4 to suppress neurite outgrowth, as well as promote neurite retraction induced by avb3-Fc, using differentiated neurons incubated with both avb3-Fc and Syndecan-4-Fc. Syndecan-4 silencing or heparitinase-pretreatment of astrocytes precluded astrocyte-mediated inhibition of neurite outgrowth. Also, neurite outgrowth over astrocytes was enhanced when b3 integrin was blocked. Furthermore, avb3-promoted neurite shortening was more rapid when co-incubated with Syndecan-4-Fc. Finally, using optical tweezers, direct interaction between Thy-1 and Syndecan-4 was demonstrated, and kinetic parameters indicated that Thy-1/avb3 binding was more stable than Thy-1/Syndecan-4 binding. These findings indicate that the combined interaction of the astrocyte avb3 integrin and Syndecan-4 with neuronal Thy-1 suppresses neurite outgrowth more effectively and accelerates the contraction of existing processes. The kinetic parameters suggest that Syndecan-4 acts as a Thy-1 co-ligand. Thus, Syndecan-4 is a cell surface component of astrocytes that contributes to the non-permissive environment for axon regeneration after brain injury.

Characterization of Mitofusin-2 knock down in Sertoli cell line and its role in phagocytosis.

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Sertoli cells have multiple roles in germ cell development, ranging from physical support to supply of nutrients. The mechanisms that regulate Sertoli cell metabolism are central to the maintenance of spermatogenesis and male fertility. One of the functions of Sertoli cells corresponds to phagocytosis of residual bodies and apoptotic spermatogenic cells. It has been shown in phagocytes that there is a correlation between mitochondrial membrane potential and the engulfment capacity. Mitochondrial dynamics and quality control includes the exchange of mitochondrial components through mitochondrial fusion and fission. 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 increase in ROS production, a decrease in mitochondrial membrane potential, higher calcium levels and a fragmented mitochondrial morphology, demonstrating an altered mitochondrial function. Phagocytosis was determined by incubating the cells with fluorescent beads and evaluated its internalization by microscopy and flow cytometry, which was reduced in a 30%. These results suggest that Mfn2 is involved in Sertoli cell phagocytosis

HPV-18 E2 protein induces partial immortalization of human keratinocytes followed by replicative senescence and modulation of the mitochondrial SncmtRNA and the ASncmtRNAs.

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Human and mouse cells express a family of mitochondrial ncRNAs. Normal proliferating cells express a sense mitochondrial ncRNA or SncmtRNA that seems to be required for cell proliferation. In contrast two antisense transcripts or ASncmtRNA-1 and -2 are downregulated in human and mouse cancer cells constituting a new hallmark of cancer. An important question is which cellular factor(s) is involved in downregulation of the ASncmtRNAs. This is indeed a difficult task and to investigate the mechanisms involve in down-regulation of the ASncmtRNAs, we studied human keratinocytes (HFK) immortalized with the complete genome of the high risk human papillomavirus HPV-18, the most representative agents of cervical cancer. Those results suggested that E2 protein of HPV18 induces downregulation of the ASncmtRNAs. However, this conclusion has to be taken carefully since those cells express other viral proteins. To confirm the role of E2 protein to induce downregulation of the ASncmtRNAs, we studied HFK cells transduced with HPV18 E2. Transduced cells became partial immortalized together with downregulation of the ASncmtRNAs. After 26 cell doubling, cells stop dividing together with downregulation of E2 protein, downregulation of the SncmtRNA, upregulation of the ASncmtRNAs together with G_2 phase exit. This work establish a new function of this viral protein.

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Characterization of a *Hexokinase-like* gene induced by root hypoxia in *Prunus* rootstocks.

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Root hypoxia in fruit trees affects growth, vegetative and reproductive development, which is reflected in low productivity, poor fruit quality, and other negative effects in trees. Most stone fruit trees (Prunus genus) are hypoxia-sensitive and for this reason they are grafted on rootstocks. We performed a large-scale transcriptome sequencing of roots from two different stone fruit rootstocks with contrasting responses to hypoxia, Mariana 2624 and Mazzard F12/1, which are tolerant and sensitive to this stress, respectively. Among flooding-responsive genes, we discovered one gene encoding a hexokinase (*HXK3-like*, ppa004715m) that was highly induced in the tolerant genotype. Hexose sugars, such as glucose and fructose are present in all plants and are the origin for most organic matter found in nature, but these hexose compounds must first be phosphorylated to be used. In plants have been identified only two families of enzymes capable of phosphorylate glucose and fructose: hexokinases (HXKs) and fructokinases (FRKs). To determine the role of Prunus HXK3-like in the context of the mechanisms of hypoxia tolerance, their function and subcellular localization was characterized. The *HXK3-like* gene was isolated and sequenced, and the structure of the codified protein was modeled. Also, we analyzed the sub-cellular localization of HXK3–GFP by transient expression in tobacco and HXK3 protein was localized or associated with the chloroplast. To analyze its role under hypoxia or other abiotic stresses, we overexpressed *HXK3-like* gene under 35S promoter in *Arabidopsis thaliana*. Additionally, we performed an *in silico* analysis of the promoter region of these two different rootstocks.

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Analysis of DcAlfin2 and DcAlfin3 transcription factors from *D.carota* as tools to increase tolerance to salinity in plants.

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The ALFIN-like transcription factors family is one of the most important in plants and are involved in various physiological processes. The first ALFIN transcription factor, Alfin1, was isolated from alfalfa, and was found predominantly expressed in roots. Transgenic alfalfa calli and plants overexpressing the Alfin1 gene were able to grow at high salt concentrations. *A. thaliana* possesses seven Alfin-like genes, where the AtAlfin5 gene is induced in abiotic stress conditions. Moreover, *A. thaliana* lines that overexpress AtAlfin5 showed a higher tolerance to these stresses.
In our group, the DcAlfin2 and DcAlfin3 genes from *D. carota* have been identified. In this work, we presented evidence of the tolerance of carrot to salt stress and the functional characterization of DcAlfin2 and DcAlfin3. These genes encode nuclear proteins that activate the transcription of reporter genes in yeast. They also bind to the promoter of the Psy2 gene of *D.carota,* which isinvolved in the synthesis of carotenoids and abscisic acid, components of an abiotic stress response pathway. We over express DcAlfin2 and DcAlfin3 genes in *A.thaliana* to evaluate the tolerance to salinity and drought *in planta*. Transgenic homozygous lines subjected to salinity treatments showed a higher tolerance respect to control plants. The higher expression levels of the DcAlfin2 and DcAlfin3 genes and the carotenoid profile correlate with the stress tolerance in transgenic lines. These results let us to propose that DcAlfin2 and DcAlfin3 genes encode for transcription factors able to promote a higher tolerance to salinity in plants.

Structure-based Discovery of New Two-Pore-Domain Potassium Channels TASK blockers.

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The two-pore domain potassium (K_{2P}) channels from TASK subfamily are highly expressed in hippocampus, cerebellum and cortical areas of the brain. Besides regulating neurotransmitter functions, TASK channels has an oncogenic potential and TASK-3 member of the subfamily is overexpressed in different types of cancer cells, for this reason the development of new selective TASK modulators could influence the pharmacological treatment of cancer and several neurological disorders. In the present work, we have searched for potential TASK blockers using a virtual screening (VS) protocol that includes pharmacophore modeling, molecular docking, and free energy calculations (MM/GBSA). One hit was identified (T3B1) inhibiting 50% of TASK channels currents with a concentration lower than 100 µM. A new derivative of T3B1 (T3B1.1) was designed rationally and synthetized. Also, T3B1.1 inhibits TASK-3 in the same concentration order. Both compounds share a pharmacophore formed by two hydrogen bond acceptor atoms and one aromatic ring. Our results are in concordance with those reported by our group previously (Kiper et al. 2015, Pflugers Arch. 467:1081-90) since the pharmacophore identified is similar to that one exhibited by other known TASK channel blockers.

Increased ER-mitochondria coupling and energy metabolism during the early phase of mitochondrial stress response.

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Introduction: Protein homeostasis is regulated by genetic programs known as 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 that support adaptation. The aim of this work was to evaluate whether the response to mitochondrial proteotoxic stress involves an early metabolic component.

Methodology: Doxycycline treatment (60 µg/mL for 0-72 h) was used to alter mitochondrial translation and induce proteotoxic stress in HeLa cells. The expression of mitochondrial and nuclear-encoded proteins was analyzed by Western blot. UPR^{mt} markers were analyzed by RT-qPCR. Oxygen consumption rate was assayed with a Clark's electrode and intracellular ATP levels were measured with a luminescence-based commercial kit. ER-mitochondria colocalization and Ca^{2+} uptake were assessed by confocal microscopy.

Results: HeLa cells treated with doxycycline showed reduced expression of mitochondria-encoded protein and increased mRNA levels of UPR^{mt} markers (CHOP, C/EBPβ, ClpP, mtHsp60) with a peak at 48 h of treatment. They also exhibited an increase in total ATP levels and oxygen consumption rates between 2 and 4 h of treatment. In addition, cells treated with doxycycline show increased ER-mitochondrial colocalization and mitochondrial Ca^{2+} uptake upon release from ER.

Conclusions: ER-mitochondria coupling and mitochondrial metabolism increase in the early phase of doxycycline-induced mitochondrial stress in HeLa cells.

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Oral Sessions 3

Gene Expression

Membrane-initiated cortisol signaling modulates the early expression of metabolism-related genes in the skeletal muscle of rainbow trout (*Oncorhynchus mykiss*).

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Cortisol is the major hormone involved in regulation of neuroendocrine stress response in teleosts. Cortisol predominantly affects target tissues through the genomic pathway, which involves interacting with cytoplasmic glucocorticoid receptors, and thereby, modulating stress-response gene expressions. Although the traditional paradigm indicates that cortisol-mediated effects are regulated by genomic mechanisms, the contribution of cortisol membrane-initiated non-genomic signaling in gene expression is unknown. In this work, we evaluated the early transcriptional response in the skeletal muscle of rainbow trout treated with cortisol or with its membrane-impermeable analog, cortisol-BSA. Juvenile rainbow trout were separated in three experimental groups and injected with cortisol, cortisol-BSA and vehicle. One hour post injection, fish were euthanized and plasma and skeletal muscle were obtained. RNA of skeletal muscle was purified and sequenced using Illumina technology and RNA-seq analysis was performed. Differential expressed genes were annotated and validated using DAVID database and qPCR, respectively. Cortisol plasma levels of experimental fish (both groups) increased 10-fold respect to control group (vehicle). 33 million of high-quality reads obtained from each condition were assembled in 74.592 transcripts. RNA-seq analysis revealed 569 genes differentially expressed in skeletal muscle in cortisol and cortisol-BSA injected fish. Glucose metabolism was the major biological process identified among those genes. The qPCR validation of candidate genes: *enolase, pyruvate dehydrogenase kinase, glycogen phosphorylase, phosphoglycerate mutase* and *malate dehydrogenase* was correlated with RNA-seq expression levels.Membrane-initiated cortisol signaling modulates the expression of glucose metabolism-related genes in rainbow trout.These results suggest that non-classic cortisol pathways contributes to regulate the stress response in fish.

Disseminated neoplasia and retrotransposons in *Mytilus chilensis.*

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Disseminated neoplasia (DN) is a proliferative cell disorder of the circulatory system of bivalve mollusks. Elucidation of the etiology of DN has been a key issue since the discovery of the disorder. The disease is transmitted between individuals and can also be induces by external agents such as bromodeoxiuridine in *Mya arenaria*. In *Mya arenaria*, we have cloned and characterized a retrotransposon named Steamer. Steamer mRNA levels and gene copy number correlates with disease status and can be used as a marker of the disease. So far the only mollusk were a retrotransposon expression relates to DN is *Mya arenaria*.

It has been reported that *Mytilus chilensis* (mejillon patagonico) can also suffers DN, we have determine that 10% of individuals collected from 4 cultured areas present morphological characteristics described in HN.

Using Steamer sequence we interrogate the transcriptome of *Mytilus chilensis,* one of the Chilean mollusks that can suffer DN. We found two putative retrotransposons, named Steamer like elements (SLEs). We verify that SLEs are indeed present in the genome of *Mytilus chilensis*. SLEs are expressed in cultured hemocytes, and their expression increase when the hemocytes are treated with bromodeoxiuridine. We are currently testing if heamocytes from DN animals have increased expression of SLEs compared to non DN animals.

Differential expression of the human T-cell leukemia virus type 1 (HTLV-1) basic leucine zipper (HBZ) isoforms is translationally controlled.

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Human T-cell leukemia virus type 1 (HTLV-1) is the etiological agent of adult T-cell leukemia (ATL) and the HTLV-1-Associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP). During its replication cycle HTLV-1 synthesizes two antisense messenger RNAs (mRNAs) that encode for two different versions of the HTLV-1 basic leucine zipper protein (HBZ). HBZ is expressed in all cases of ATL and is directly associated with virus pathogenicity. The spliced (*sHBZ*) and the unspliced (*usHBZ*) versions of the *HBZ* mRNA differ in their 5'untranslated regions (5'UTR) and the proteins they encode, sHBZ and usHBZ, are differentially expressed in cells, where the sHBZ protein is more abundant. Here, we show that differential expression of the HBZ isoforms is under translational control. Translation initiation of *sHBZ* mRNA uses internal initiation (sHBZ-IRES) and is more efficient than that of *usHBZ* mRNA, which relies on a cap-dependent mechanism. Additionally, the sHBZ-IRES relies on the ribosomal protein S25 (RPS25) and is sensitive to edeine, which suggest that the recognition of the initiation codon requires scanning. Taken together, these findings demonstrate that differential expression of the HBZ isoforms is subjected to translational control.

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DISC1 regulates translation and Stress Granule dynamics in an Akt/mTORC1-independent manner.

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The Akt/mTORC1 pathway has been widely studied in cancer due to its relevance in promoting cell growth and proliferation. It relies on stimulation of anabolic processes like protein synthesis through phosphorylation of targets. Given its importance on cellular homeostasis, Akt/mTORC1 activity is acutely regulated according to energy and nutrient availability, and growth factors stimulation. DISC1 is a negative regulator of the Akt/mTORC1 pathway, and mutations of DISC1 are associated with several pathologies of the central nervous system. DISC1 regulates neuronal growth, migration and differentiation through its inhibitory interaction with Girdin, a strong activator of Akt/mTORC1. Thus, DISC1 is expected to inhibit translation through this mechanism. However, DISC1 has also been found to interact with some mRNAs and eIF3, a key component of the translational machinery, suggesting that it could control translation independently of Akt/mTORC1. Here, we studied this possibility through depletion of DISC1 either by shRNA or CRISPR/Cas9-mediated genome editing in Tsc2-/- cells, in which mTORC1 activity cannot be enhanced by DISC1 loss of function. We preciously found that DISC1 regulates translation and stress granule(SG) assembly during arsenite-induced oxidative stress. Now we show that DISC1 also promotes translation in cells overexpressing TIA-1 (a SG nucleating protein) even in the absence of any other stress. Through Super Resolution Structured Illumination Microscopy we show that DISC1 locates outside of SG, suggesting a mechanism by which DISC1 is regulating translation. Our studies identified a novel mechanism by which DISC1 could control translation, with potential implications in central nervous system disorders or even cancer.

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Asociation of SetDB1 to ribosomes during the cell cycle and its impacts on the H3K9me1 mark.

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Previous studies characterized the maturation cascade involved in the establishment of post-translational modifications and folding of newly synthesized histones H3 and H4. On this maturation cascade, the first methylation mark imposed is H3K9me1, which occurs cotranslationally by SetDB1, a methyltransferase associated to the ribosome. However, only 36% of the soluble H3 carries this modification, which suggests the existence of a regulatory mechanism for the generation of this mark. Using synchronized cells, we performed Western Blot analyses to assess the presence of SetDB1, H3, and H3K9me1 on ribosomes obtained from cells enriched at different stages of the cell cycle. We found that SetDB1 is enriched on ribosomes at late S phase. We observed an accumulation of H3 and H3K9me1 at early and late S phases. We conclude that there is a cell cycle dependent regulatory mechanism for the generation of H3K9me1. Future studies will focus into identify the molecular basis of this process.
FONDECYT 1160480 and Basal Project PFB-16.

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

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Human Immunodeficiency Virus type-1 (HIV-1) gene expression involves the synthesis of a complex transcriptome including a subset of completely and incompletely spliced transcripts and one unspliced mRNA molecule. As cellular mRNAs, completely spliced transcripts follow the classical gene expression pathway in which nuclear export and translation are strongly stimulated by splicing. In contrast, the HIV-1 unspliced mRNA does not benefit from splicing and it is retained and degraded in the cell nucleus unless the viral protein Rev is present. Here, we confirm that the recruitment of Rev to the unspliced mRNA is sufficient to substitute the effects of splicing on nuclear export and translation. Interestingly, these functions of Rev are interconnected since no effect of Rev on translation was observed when the unspliced mRNA is exported through NXF1. We also demonstrate that Rev interacts with the DEAD-box RNA helicase eIF4A favouring the recruitment of the RNA helicase to the unspliced mRNA. Together our data reveal a novel mechanism by which Rev interconnects nuclear export and translation of the unspliced mRNA in order to ensure efficient Gag synthesis during viral replication.

Molecular regulation of HIG2A, HIG-1 hypoxia inducible domain family member 2A, a protein mediator of the respiratory chain supercomplex assembly.

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Introduction: HIG2A promotes cell survival under hypoxia and mediates the assembly of electron transport chain complexes into respiratory supercomplexes in the inner mitochondrial membrane. Supercomplexes assembly is correlated with mitochondrial morphology and bioenergetics. In this regard, the interplay between mitochondrial fusion and fission, called mitochondrial dynamics, might be involved in. Our general goal is to study the function of HIG2A in the regulation of mitochondrial physiology and dynamics.

Methodology: *HIGD2A* gene expression was analyzed by RT-qPCR in HEK293 and C2C12 cell lines and in C57BL/6 mice. Transcriptional regulation was also studied by *HIGD2A* promoter reporter expression and chromatin immunoprecipitation (ChIP)-qPCR assays. Subcellular localization of HIG2A was analyzed by immunocytochemistry and Western blot.

Results: *HIGD2A* gene expression is positively regulated by hypoxia and glucose availability, and dependent on lipid metabolism and the cell cycle-related transcription factors PPARα and E2F1, respectively. E2F1 was found to bind the promoter region of *HIGD2A*. Differential expression of *HIGD2A* gene was found in mice depending on tissue, age and cellular metabolism. *HIGD2A* knockdown SY5Y neuroblastoma cells displayed a decrease in mitochondrial fusion. On the other hand, the depletion of *HIGD2A* in HEK293 cells by CRISPR/Cas9 system showed an increase in the protein expression of complex III and the mitochondrial dynamics protein OPA1 while the fusion protein MFN2 was decreased. At protein level, HIG2A was mainly localized in mitochondria and its expression, increased by hypoxia.

Conclusions: HIG2A protein might function as a regulator of respiratory supercomplexes assembly in response to hypoxia and changes in cellular metabolism.

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MicroRNA-335-5p and extracellular vesicles in gastric cancer: from *in vitro* to *in vivo* functional studies.

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Introduction: We have previously shown the downregulation of miR-335-5p in gastric cancer (GC) tissues and demonstrated that miR-335-5p overexpression correlates with an inhibition of migration, invasion, proliferation and clonogenic capacities. To further evaluate the role of miR-335-5p, we aimed to investigate expression of miR-335-5p in GC derived extracellular vesicles (EVs), behavior of these vesicles on the cell invasiveness and their role *in vivo*.

Methods: EVs were isolated from supernatants from GC cell lines and cells transfected with miR-335-5p mimics. MiR-335-5p expression levels in cell lines and their EVs were analyzed by qPCR. First, the invasive properties of cells transfected with miR-335-5p mimics, and cells treated with EVs overexpressing miR-335-5p were studied. Next, GC cells were intraperitoneally injected into mice to create an *in vivo* model of GC tumor. The EVs were labeled with fluorescent dye DiR, injected intravenously in the tail of mice and their distribution in time was evaluated using In Vivo visualizing machine.

Results: Overexpression of miR-335-5p and treatment of cells with EVs overexpressing miR-335-5p was correlated with decreased invasive properties. Next, the model of intraperitoneal tumor formation was established showing also formation of metastasis in various organs. The intravenous injection of DiR labeled EVs derived from untreated cells and cells overexpressing miR-335-5p showed different distribution *in vivo*. Interestingly, EVs with miR-335-5p were preferably delivered to stomach and were even able cross the encephalitic barrier.
Conclusion: These data complement the clinical relevance of miR-335-5p and provide further evidence to support its potential role in metastatic tumor suppression.

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Oral Sessions 4

Microbiology and Immunology

FUN-LOV: Fungal LOV domains for optogenetic control of heterologous protein expression and flocculation.

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Optogenetic switches promote gene expression upon light stimulation. These synthetic switches have become in a powerful tool for gene expression, with potential uses in heterologous protein expression and phenotypes control, overcoming the obstacles of chemical inducers and replacing its uses by light. In this work, we implemented in yeast an optogenetic system based on the interaction of two blue-light photoreceptors from *Neurospora crassa*, WC-1 and VVD, which we called FUN-LOV system. The FUN-LOV showed over 1300-fold of induction for the luciferase reporter gene upon blue-light or white-light stimulation, and with a highly dynamic and temporal resolution. We used the FUN-LOV system for heterologous expression of the biotechnologically relevant enzyme, limonene synthase. Western blot analysis showed over a 100-fold of protein induction upon light activation, representing 2.5 times more expression than chemical inducer activation. Additionally, we used the FUN-LOV system to control the expression of genes related with yeast flocculation (*FLO1*, *FLO11* and *TUP1*). Depending of the target gene controlled by the FUN-LOV system, Flocculation in Light (FIL) or Flocculation in Darkness (FID) were achieved. The light controlled expression of *FLO1* showed FIL phenotype, whereas the light controlled expression of *TUP1* showed FID phenotype. Overall, the results confirmed the potential of this optogenetic tool to control two biotechnologically relevant phenotypes, heterologous protein expression and flocculation, which finally results in the development of new yeast strains with industrial applications.

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Genetic Polymorphisms of interferon lambda (IFN-λ/IL28B) and tumor necrosis alpha (TNF-α) modulate clinical severity of respiratory syncytial virus bronchiolitis in pediatric patients.

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Respiratory syncytial virus (RSV) is the leading cause of respiratory tract infection in infants. In this study we evaluated if single nucleotide polymorphism (SNPs) associated to the level of expression of interferon lambda (IFN-λ/IL28B), rs12979860 (CT/TT/CC) and rs8099917 (TG/GG/TT), and the tumor necrosis Factor (TNF-α), rs1800629 (AA/AG/GG), link with the severity of RSV bronchiolitis in a pediatric population. For this, 629 infants with no comorbilities were prospectively enrolled between July 2014 and December 2016 at admission time to the Emergency room, ambulatory and pediatric section of the *Hospital Clínico Universidad Católica* or *Hospital Doctor Sótero Del Río*. Biological samples (buccal swabs) were categorized according to the severity of RSV induced bronchiolitis exhibited by the donor. Genomic DNA was extracted from samples and SNPs of IL28B (rs12379860/rs8099917) and TNF-α (rs1800629) were determined using TaqMan technology. Results show that non-severe bronchiolitis patients associated to a major proportion of eutocic delivery, less admission to intensive care unit, reduced mechanical ventilation, a diminished bacterial infection, shorter hospital and ICU length-of-stay and less days of supplemental oxygen requirement. An association between the homozygous status of the minor allele of IL28B SNP rs8099917 (GG) with lower clinical severity (Odd Ratio: 0.13; P = 0.038) was evidenced, while the major allele (GT/TT) linked to severe cases. No association was observed between TNF-α rs1800629 or IL28B rs12379860 and the severity of RSV induced bronchiolitis. Based on these findings we conclude that IL28B SNP rs8099917, but not IL28B rs12379860 or TNF-α rs1800629, links with the clinical severity of RSV bronchiolitis.

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Heme Oxygenase-1 reduces the viral replication and lung diseases after Human Respiratory Syncytial Virus infection.

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Human respiratory syncytial virus (hRSV) is the major cause of severe lower respiratory tract infections in children. Here, we examined the effects of inducing the activity of the host enzyme heme oxygenase-1 (HO-1) as a possible antiviral strategy against hRSV infection.

Methods: A549 cells were infected with hRSV in the presence or absence of CoPP (HO-1 inductor), SnPP (HO-1inhibitor), or vehicle control for 24 h and then cells were analyzed for measure infection. For in vivo experiments, BALB/cJ mice were treated for 24 h with CoPP, SnPP or vehicle control and then intranasally inoculated with either mock or hRSV (1x106 PFU). Disease progression was monitored by animal weight loss during 4 days. Mice were euthanized and BALF was analyzed for neutrophil infiltration by flow cytometry and lung homogenates of each experimental group of infected mice, were collected and quantified for viral copy number. The same methodology was performed in transgenic mice (tTA-HO-1) that overexpress human HO-1 in in MHC-II$^+$ cells.

Results: Our results show that HO-1 induction reduced the loss of body weight due hRSV-induced disease. Further, HO-1 induction also decreased viral replication and lung inflammation, as evidenced by a significant reduction in neutrophil infiltration to the airways. Furthermore, similar antiviral and protective effects were observed by inducing the transgenic expression of human HO-1 in MHC-II+ cells in mice. Finally, in vitro data suggest that HO-1 induction can reduce airway epithelial cell permissivity to the virus.

Conclusion: HO-1 induction could protect the host from the pulmonary pathology developed upon hRSV infection.

CONICYT, Beca Doctorado Nacional 2013 No. 21130507 Millennium Institute on Immunology and Immunotherapy.

Transcriptional changes induced by Infectious Pancreatic Necrosis on the *Salmon Salar* head-kidney.

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Salmon is one of the most important species for aquaculture in the world. Its intensive farming, as with other species, face sanitary problems leading to large economic loss and limit the quality of the final product. Infectious pancreatic necrosis (IPN) is one of the most serious viral disease affecting early stages of salmonids causing high mortality and morbidity. In *Salmon salar*, outbreaks of IPN occurs typically during newly-hatched fry and post-smolt stage. In this work, we studied the transcriptional changes produced by IPNv on post-smolt *Salmon salar*. Focusing on a haematopoietic-lymphoid organ, we dissected head-kidney from healthy and IPNv-infected fish. The transcriptomic profile was obtained by RNAseq and changes on expression were determined by an established bioinformatics pipeline. We observed more than 6000 genes expressed in head-kidney, from which 160 were differentially expressed. Interestingly, from these genes over 100 of them were downregulated. Through a pathway enrichment analysis, we observed that many components of the innate immune response were dramatically downregulated. Genes such as *timp2a*, *stbd1*, *leukocyte elastase inhibitor-like*, and *gyg1b* were affected. These results will help to deeply characterize the immune responses of Salmon salar during viral infection as well as to understand the influence of IPNv on host's transcriptome.

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The NSs protein from the Andes Virus inhibits the type I IFN response pathway.

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Andes virus (ANDV) is a rodent-borne hantavirus member of the *Bunyaviridae* family of viruses. ANDV is endemic in Argentina and Chile and is the major etiological agent of hantavirus cardiopulmonary syndrome (HCPS) in south America. ANDV features a tripartite genome consisting of three negative polarity single-stranded RNA segments designated large (L), medium(M) and small (S), packed into helical nucleocapsids.The L and M messenger RNAs (mRNAs) encode the RNA polymerase and a glycoproteinprecursor that is co-translationally processed to yield two envelopeglycoproteins (Gc and Gn), respectively. The SmRNA encodes the nucleocapsid (N) protein and the NSs protein from an overlapping (+1) open reading frame. At early stages of infection ANDV inhibits the cellular type I IFN response, through a yet not fully understood mechanism. The ANDV Gn and N proteins have been shown to inhibit the type I IFN pathway by blocking its signaling at level of TANK-binding kinase 1 (TBK1). TBK1 is involved in the activation of NFkB and IRF-3 which in turns triggers transcription of the IFN genes. Here, we use an assay in which the luciferase reporter is under the control of a minimal IFN-b promoter to demonstrate that the ANDV-NSs protein blocks the activation of the IFN-b response pathway. Thus, findings reveal a role for the ANDV-NSs protein in the evasion of the cellular innate immunity response.

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Hantavirus receptors expression in a lethal pulmonary syndrome animal model.

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Hantaviruses can cause hemorrhagic fever with renal syndrome (HFRS) and the highly lethal hantavirus pulmonary syndrome (HPS) in humans, being Hantaan and Andes the respective prototype viruses. Both diseases are related with an increased vascular permeability; yet several pathogenesis factors have been suggested. Actually, one of them may be a differential receptor usage; among them the b3 integrin subunit and the receptor for the globular head domain of complement C1q (gC1qR) are used by pathogenic hantaviruses *in vitro*. In this work, we analyze the expression of b3 integrin and gC1qR in an animal model for HPS. For this purpose, we performed immunohistochemical and qPCR studies of lungs and kidneys at 8 and 12 days post-infection (d.p.i.) with Andes hantavirus. In control and 8 d.p.i animals, both receptors are localized in pulmonary veins while in 12 d.p.i. moribund animals the lungs exhibit strong features of edema accompanied by weaker receptors staining. Interestingly, the qPCR results show that the expression of b3 integrin and gC1qR is reduced in lungs of 8 d.p.i. animals, even though the histology of the tissue appears normal with no signs of disease. The decrease of receptors mRNA levels in the lungs at 8 d.p.i. seems to be highly specific since infected kidneys of the same animals show similar receptor mRNA levels compared to controls. Together, these results suggest that the reduced expression of b3 integrin and gC1qr receptors may be an important initial step involved in vascular permeability and subsequent pathogenesis.

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Proteomics characterization of *Piscirickettsia salmonis* LF89 strain, in liquid medium AUSTRAL Broth.

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Introduction: *Piscirickettsia salmonis* is the predominant bacterial pathogen affecting the Chilean salmonid industry. This bacterium is the etiological agent of piscirickettsiosis, a significant fish disease. The efforts have not been efficient to be able to characterize proteomically this bacterium.

Methods: The P. salmonis LF-89 (equivalent to ATCC VR-1361) type strain was grown on AUSTRAL broth at 18 °C for exponential phase. Proteins were digest by trypsin and peptides were subjected to a two-dimensional chromatography coupled to a mass spectrometer LTQ Velos pro. And the bioinformatics analyzes were performed using the SEQUEST algorithm and the proteins were annotated against the GO database (gene ontology)

Results: We have identified 1537 proteins using (multidimensional identification technology) MudPit, by LC-MS/MS. Using gene ontology we have grouped the proteins by compartment, biological processes and molecular function. Where 60% of the proteins are involved in metabolic processes, followed by transport and regulation with 10%. Additionally we identified proteins corresponding to pathogenicity factors such as the type IV secretion system, flagellum, and antibiotic resistance proteins. The heat shock protein HSP60 or GroEL, a known chaperone, is the most abundant protein. This protein was found in outer membrane, periplasm, internal membrane and cytoplasm.

Conclusion: This is the most complete study carried out in *P. salmonis*, from the proteomic point of view, and obtaining a total characterization of the behavior of this bacterium in an exponential growth phase. This type of proteomic studies are key for the search of new therapeutic and effective targets against *P. salmonis.*

Austral-omics, facultad de Ciencias, DID-UACh

Use of *Botrytis aclada* laccase for the inactivation of tetracycline antibiotics.

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Laccases are multicopper oxidases that use oxygen and produce water as byproduct for oxidizing a great variety of organic substrates. This has made them useful in several industrial applications and more recently in the inactivation of antibiotics. Environmental contamination by antibiotics is important due to the fact that they may cause selective pressure for rising antibiotic resistance in bacteria. Tetracyclines are broad-spectrum antimicrobial agents widely present in wastewater because conventional water treatment methods are not able to remove them. Although the inactivation of antibiotics of the tetracycline family has been reported for different laccases, most of these studies have focused on engineering aspects paying little attention to the structural determinants of the laccase catalytic efficiency. In the present work, we aim to fill this gap by using the laccase from the plant pathogen *Botrytis aclada* as a model. This enzyme presents one of the highest expression levels and chloride tolerance reported for a laccase family member and its crystallographic structure has been solved. We modelled the enzyme interaction with tetracycline and oxytetracycline by homology modeling, molecular dynamics simulation and docking. We also cloned and expressed the Botrytis aclada laccase in *Pichia pastoris* and purified the enzyme in two chromatographic steps. We evaluated the inactivation of the antibiotics at different treatment times by quantifying the effect of the reaction products over the growth of *E. coli* cultures. We observed a slow time course of inactivation along 48h treatment. Furthermore, we also observed changes in the absorbance spectrum of the treated samples.

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Oral Sessions 5

Protein Structure and Function

Critical residue interactions in the hantavirus Gc post-fusion trimer.

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Rodent-transmitted hantaviruses are human pathogens,whichin the case of Andes virus can reach fatality rates of 50%. They enter cells via receptor-mediated endocytosis, and the acidic pH of the endosomes activates the fusogenic activity of the viral envelope protein Gc, which drives fusion of viral and endosomal membranes for entry. Recent structural studies have demonstrated that Gc is a class II fusion protein, with three characteristic beta-sheet rich domains: I, II and III. The post-fusion conformation shows multiple polar and electrostatic interactions stabilizing the post-fusion trimer. We show here that mutating the residues involved in the intra- and inter-chain interactions of domain I within the trimer lead to a drastically destabilized trimer and its fusion activity is abrogated, suggesting that Gc is arrested in an intermediate preceding the stable post-fusion conformation. In contrast, similar mutations affecting inter-chain interactions in domain II do not have an impact in trimer stability, and the fusion activity is maintained.

Taken together, our results suggest that within the Gc post-fusion trimer, the electrostatic intra- and inter-chain interactions of domain I but not inter-chain contacts between domains II are critical for achieving the stable post-fusion structure required to release enough energy to drive membrane fusion.

FONDECYT 1140050 -Basal PFB-16

Understanding the Covalent binding of Clavulanate against β-lactamases (TEM-1 and KPC-2) with QM/MM methods.

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β-lactamases are a primary cause of bacterial resistance to β-lactam antibiotics for many important human pathogens (particularly Gram-negative bacteria) (Hermann, Pradon, Harvey, & Mulholland, 2009). Inhibitors of β-lactamase have been implemented as a dual therapy with antibiotics, but there are only four inhibitors clinically approved and resistance to these compounds is also rising (Worthington & Melander, 2013). For β-lactam inhibitors, after acylation, the opening of five-membered ring leads to the formation of a transient imine intermediate then it rearranges several times to form a *trans* or *cis* final enamine inhibition-products. Slow hydrolysis of this product by the enzyme leads to an inhibited β-lactamase (Drawz & Bonomo, 2010).

A computational study of reaction mechanism for the first step on the deacylation of the inhibitor clavulanate with TEM-1 (inhibited) and KPC-2 (hydrolyzed) enzymes (Papp-Wallace *et al.*, 2010) using QM/MM Umbrella Sampling with DFTB method is presented. 2D free energies surfaces for the reactions were calculated using the weighted histogram analysis method (WHAM) and the minimum energy path (MEP) was identified; where the highest point along the MEP is taken as the transition state giving the activation free energy "ΔG_{calc}".Our results show that TEM-1 and KPC-2 have an approximate 5 kcal/mol difference in ΔG_{calc}. Such results are in good agreement with inhibition experimental data for two enzymes in which KPC-2 is less inhibited by clavulanate than TEM-1. We hope our methodology can assist the design and development of covalent inhibitors through a computational screening of inhibitory activity of other molecules.

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A combination of computational and experimental approaches reveals differential stability patterns within the fold-switching domain of RfaH.

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Unlike most polymers, proteins commonly are found in a highly-structured native state, a minimum in its energetic landscape sufficiently low to be stable and broad to allow for function-related motions. Yet, metamorphic proteins may fold into at least two structurally and functionally distinct stable configurations, challenging this broadly accepted paradigm. Such is the case of the transcriptional regulator RfaH, which exist in an auto-inhibited state where its C-terminal domain (CTD) is folded as an α-helical hairpin preventing RNApol binding, but upon recruitment to the transcriptional machinery it turns to its active state in which its CTD is dissociated and refolded as a standalone β-barrel, allowing RfaH to form a processivity clamp around the DNA and synchronize translational processes. Combining hydrogen-deuterium exchange mass spectrometry to calculate the opening constant (Kap) of peptides and a molecular dynamics confinement-conversion-release strategy to estimate free-energy decomposition, we determined the local stability of regions comprising RfaH CTD in both states with excellent agreement between experiments and simulations. These results show that β-CTD is monotonously stable with only loops displaying higher flexibility, while α-CTD shows anisotropic stability particularly in the region comprising residues 127-141 forming the tip of the hairpin, displaying lower Kap than its equivalent in β-CTD, while the rest of the hairpin exhibits Kap at least one order of magnitude higher. The existence of clearly-defined stabilizing regions towads each state reveals the conflicting nature of RfaH, shedding light on the sequence determinants that encode the driving forces responsible for its structural duality.

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Mechanism of adaptation to hypersaline environments and its evolutionary history in ADP-dependent phosphofructokinases from the order *Methanosarcinales.*

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Halophilic organisms inhabit hipersaline environments where large osmotic pressure has driven the evolution of molecular mechanisms of adaptation. Although halophilic organisms are present in three phylogenetic groups of archaea, only the orders *Halobacteriales* and the *Methanosarcinales* have cultivable organisms.
Halobacteriales proteins are known to carry out most of their functions in the presence of molar concentrations of salt. Previous studies demonstrated that proteins from these organisms harbor a different amino acid composition, characterized by an increase in negative charged residues and a decrease in large hydrophobic residues. However, studies evaluating the hypersaline adaptation mechanism of *Methanosarcinales* proteins are absent.

To address this question the ADP-dependent phosphofructokinase family (ADP-PFK) from *Methanosarcinales* was studied. Reconstruction and characterization of the last common ancestor of ADP-PFK from *Methanosarcinales* suggests that the halophilic character is an ancestral trait. Additionally, bioinformatic analysis of amino acid composition of *Methanosarcinales* proteins shows that their share a composition similar to the one observed in *Halobacteriales* proteins, differing only in a few residues (Ala, Ile, Lys). Measurements of enzyme activity in the presence of salts shows that *Methanosarcinales* enzymes from both halophilic and mesophilic models are moderately inhibited, displaying a similar behavior. Nevertheless, their inhibition was reverted in the presence of betaine, a protective osmolite. Our results suggest that *Methanosarcinales* proteins adapt to saline environments through a mechanism similar to the one presented by *Halobacteriales* proteins. This mechanism is conserved throughout the evolution and is complemented with compatible solutes.

Engineering the cofactor specificity from NADH to NADPH of the D-lactate dehydrogenase from *Escherichia coli.*

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Under anaerobic conditions and in the absence of electron acceptors, *Escherichia coli* converts glucose to a mixture of fermentation products. One of these products is lactate which is generated by the reduction of pyruvate with the concomitant oxidation of NADH. This reaction, catalyzed by the D-lactate dehydrogenase (LdhA), allows the organism to restore NAD, an important input for glycolysis. In this work, we used structural and evolutionary tools for engineering the cofactor specificity of LdhA to improve the lactate production in *E. coli* by coupling it to the oxidation of NADPH. The enzyme belongs to the 2-hydroxyacid dehydrogenase (2HADH) family, which consists of D-isomer-stereospecific enzymes with a Rossmann fold domain that binds NAD(P)(H). Sequence traits for cofactor preference were found in the residues of the β7-α7 loop of members of the 2HADH family. In that sense, LdhA from *E. coli* presents residues in this loop which favors the NADH affinity, and the kinetic characterization indicates that the enzyme is 160 fold more specific for NADH but possesses a similar K_M for Pyruvate with both cofactors. To allow the binding of NADPH, we mutated residues of the β7-α7 loop for those found in NADP-binding homologous. We obtained an NADPH-specific enzyme with 57-fold preference over NADH. The increment of the specificity constant for NADPH in the mutant enzyme was 9164-fold over the wild type form.

Beca Doctorado Nacional, Proyecto VID ENL012/16, CINV Milenium Institute

Dynamics of the dimeric FoxP1 at single-molecule level.

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Domain swapping has shown to be a relevant folding and oligomerization process in several proteins, in which two or more subunits exchange specific secondary structure segments to obtain intertwined species. The functional relevance of this mechanism has been described in widely studied proteins as p13suc1 and diphtheria toxin, where thermodynamic and kinetic behavior of this mechanism has showed that the unfolded state is a requirement to obtain the domain-swapped conformation.

Previous findings have shown that DNA-binding domain of human FoxP proteins reach a dimeric structure via domain swapping. Specifically, we have obtained relevant biophysical ensemble details related to this process using FoxP1 as model, which present highly flexible regions that are locally unfolded when the monomeric intermediate is formed. To obtain a detailed structural description of the domain-swapped dimer of FoxP1, we introduced cysteine residues in specific regions to attach fluorophores andstudied the dynamic and sub ensembles conformations via single-molecule FRET using the multiparameter fluorescence detection (MFD) approach. Our data showed that two conformations of the dimeric protein were observed: a closed sub-ensemble consistent with the domain-swapped FoxP1, and an open sub-ensemble whose distances and dynamic possibly corresponds to an unstructured and flexible protein. On the other hand, we studied the denaturant effect in sub conformational transitions, showing that dynamics of the most stable region is the limiting step of the dissociation. These findings will be relevant to understand the kinetic and thermodynamic properties of this protein described in ensemble conditions.

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Determining the knotting pathway of MJ0366 by using different pulling geometries in optical tweezers.

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Knotted proteins constitute a small group of proteins whose main chain entangle to form a knot. However, is not clear how they thread their polypeptide chain and neither the energetic cost associated to this process. To address these questions, we used optical tweezers to mechanically unfold the trefoil knotted protein MJ0366 from *Methanocaldococcus jannaschii.*

On one hand, when the knot is tightened (pulling C and N terminus), MJ0366 displays single unfolding/refolding transitions with a molecular extension consistent with a two-state mechanism of unfolding. The application of the Crooks theorem predicts a value of ΔG_{kn} = ~13 kcal/mol between the folded and unfolded state, both knotted.
On the other hand, when the N-terminus is not allowed to thread the protein (pulling the N-terminal and the threading loop), MJ0366 still fold correctly, although the refolding transitions are missing. To determine protein stability (ΔG_{uk}),we calculated the folding rate from the probability of refolding as a function of the waiting time at different low forces, and the unfolding rates from the Dudko's analysis of unfolding force distributions. The value of ΔG_{uk} is ~4 kcal/mol for the equilibria between the fully unfolded/unknotted and the native state of MJ0366. Notably, when C-terminus is not allowed to thread the chain (pulling the C-terminal and the threading loop) the protein is unable to fold and remain trapped as a misfolded state. Therefore, our results indicate that the folding route of MJ0366 occurs by threading the C-terminus and requires around 9 kcal/mol to form the knot (ΔG_{k-} ΔG_{uk}).

In silico and experimental structural data of γ^{33} subunit associated to R-phycoerythrin from *Gracilaria chilensis* (Rhodophyta: Gracilariaceae).

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Phycobilisomes (PBS) are accessory light harvesting protein complexes formed mainly by phycobiliproteins (PBPs). The PBPs absorb light being efficiently transferred to reaction centers due to chromophores covalently bond to specific cystein residues in protein sequences. Additional to phycobiliproteins, there are linker proteins responsible for assembly, stabilization and tuning of energy transfer between chromophores of PBS. The linker (γ^{33}) subunit from *Gracilaria chilensis*, is a chromophorylated rod linker associated to $(\alpha\beta)_6$ hexamers of R-phycoerythrin (R-PE). Their role in the energy transfer process is not clear yet. The cloning and sequencing of γ^{33} coding gene was carried out. Prediction servers were used to analyze the *in silico* translated sequences and the prediction of secondary structure. The protein was purified and the sequence was confirmed by mass spectrometry. α-helix secondary structure was confirmed by circular dichroism. The sequence was organized in two homologous domains. Conservation of cysteines indicate possible chromophorylation sites. Spectroscopic assay suggests that one phycourobilin chromophore was double bonded, and mass spectrometry analysis identified these binding sites as Cys62 and Cys73. A preliminar molecular model of γ^{33} subunit was built using *ab initio* methods. The model revealed that the cysteines involved in the double bond are located in a helical region, remembering double bond conformation of phycourobilins on β subunit of R-phycoerythrin.

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New Members Session 1

CLK-peptides as superior surface stabilizers for silver nano structures: Role of peptide chain length and applications in nanomedicine.

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Three new collagen mimetic peptides containing the CLK motif as anchoring arms were tested for silver nanoparticle surface stabilization, both using experimental techniques and enhanced sampling molecular simulations. Our data indicate that peptide length does have an important effect in the resulting nanosilver's colloidal stability and biological performance. These new materials have shown great effectiveness in the healing of tissues such as skin, retina, and heart, among others.

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Structural similarities between SMCT1 and NIS: Clues to the origin of apical iodide transport in the thyroid.

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Several apical iodide translocation pathways have been proposed for iodide efflux into thyroid follicular cells, including a pathway mediated by the sodium-coupled monocarboxylate transporter 1 (SMCT1). The role of SMCT1 as iodide transporter remains controversial, because expression studies with *Xenopus oocytes* demonstrated that SMCT1 does not cotransport iodide or other inorganic anions; however, subsequent analyses have revealed iodide transport by SMCT1 at low extracellular sodium concentrations. Herein, we evaluate structural and functional similarities between SMCT1 and the well-known sodium-iodide symporter (NIS) in the thyroid. Comparative models of hNIS and hSMCT1, based on the structure of the galactose transporter vSGLT, predict a conserved sodium binding motif characteristic of sodium-dependent transporters. Also, we identify a conserved iodide-binding pocket, comprising a region previously found to be mutated in NIS for patients with hypothyroidism. In hNIS, G93 is critical for iodide permeation and interestingly, in hSMCT1 the corresponding residue is replaced by threonine. Free energy calculations using polarizable molecular dynamics models reveal a strong iodide affinity in this region for wild-type hNIS, which is reduced for the G93T hNIS mutant, consistent with experimental evidence. Wild-type hSMCT1 contained a similar iodide-binding pocket, although with less affinity than wild-type hNIS. The affinity was strengthened in the T91G hSMCT1 mutant. Analysis of these simulations revealed that a conserved tryptophan residue (W255 in hNIS and W253 in hSMCT1) plays a major role in iodide binding. Our results provide further evidence of similar structural and functional elements between hSMCT1 and hNIS.

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The healthy aging transcriptome of *Drosophila melanogaster.*

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A major challenge is to understand which cues contribute to the appearance of the hallmarks of aging.Transcriptional alterations constitute one primary hallmark of aging. To gain insights into this matter we used *Drosophila* because it allows whole organism time course transcriptomics of a collection of healthy individuals raised synchronically in an identical environment. We performed bioinformatics in the transcriptomes of all stages of the lifespan of the fruitfly to discover gene networks (GNs) acting during healthy aging. We validated these data with molecular tools. GNs expression levels account for the state of pathways known to be dysregulated during aging. However these GNs account for some but not all of the hallmarks of aging. To discover new interactions among the differentially expressed transcripts in aging we obtained gene co-expression modules which reveal new age and sex specific genetic interactions during the aging process. Among the differentially expressed transcripts in aging, we discovered too a set of long non-coding RNAs (lncRNAs). We mapped these lncRNAs to the modules and characterized its nearest coding gene to infer their putative function. A few lncRNA are common to both sexes. Such lncRNAs are positionally conserved to the human genome and their nearest coding RNA are expressed in brain tissue pointing to a role of lncRNAs in the aging brain. These data help elucidate the cues that lead to the appearance of the hallmarks of aging and thus contribute to the understanding of how a “healthy aged phenotype” is achieved.

FONDECYT 11130203.

Levels t6A modification modulate protein homeostasis, TOR kinase activity and cell growth.

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N6-threonylcarbamoyl-adenosine (t6A) modification occurs at position 37 of tRNAs that decode A-starting codons, including the eukaryotic initiator tRNA (tRNAiMet). Yeasts lacking t6A synthesis machinery, such as mutants for Tcs3p (Kae1p) or Tcs5p (Bud32p), show slow-growth phenotypes. Here we describe the existence of KEOPS complex in Drosophila and, for the first time in a non-microbe, we show that KEOPS is required for t6A synthesis. As in yeast, deficiencies of KEOPS´ components lead to severe reductions in cell and animal size. Moreover, just the proportion of t6A-modified tRNAiMet behaves as a restrictive factor for growth. Accordingly altering the fraction of t6A-modified tRNAiMet, by expressing an unmodifiable tRNAiMet or changing the levels of Tcs3, regulates TOR activity and influences cell and animal growth in vivo. In addition, the subsistence of eye proliferating cells and their translational profile depend on specific amounts of t6A-modified tRNAs. These findings reveal an unprecedented relationship between the proteome, the translation machinery and TOR kinase, where translation selectivity and efficiency, limited by the availability of t6A-modified tRNA, assist to define cellular proteome and TOR activity, ultimately controlling growth potential in eukaryotic cells.

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New Members Session 2

SENP6: The master SUMO chain breaker.

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The SUMO protease SENP6 is a key SUMO deconjugase controlling the cellular levels of the proto oncogene PML as well as play an important role in DNA repair. We previosly reported that the turnover of the STUBL ligase RNF4 is regulate by SUMO chains trogh and activation mechanims in cells and in vitro. As follow up of the previous work, here we show that the levels of SUMO bodies induced after SENP6 ablation is induce by replicative stress agents such as aphidicolin or Hydroxyurea, evenmore interestingly, in SENP6 deplete cells, SUMO bodies shown a temporo-spatial simetric localization which is transmited to the daugther cells in G1. We found a correlation between SUMO bodies and 53BP1 G1 bodies, therefore recruitment we used CRISPR/Cas9 technology (Cas9 D10A nickase) to generate an heterocygote 53BP1 endogenously tagged with YFP cell line. Finally, we implemented a new mass spectrometry strategy to identified the responsable signalling for the uncontrolled increase of SUMO conjugation upon SENP6 ablation. In summary SENP6 ablation impact the DNA repair reponse and triggers dramatice mitotic aberrations that lead into cell dead by a dramatic and uncontrol accumulation of SUMO chain.

Fondo de innovacion para la competitividad FIC16-19, Codigo BIP 30470186-0.

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Lysyl Oxidase Like-2 Crosslinks Collagen IV of Glomerular Basement Membrane.

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Introduction: The 7S dodecamer is recognized as an important structural crosslinking domain of collagen IV networks that provide mechanical stability to basement membranes, essential for the development and maintenance of tissue architecture. Although the 7S dodecamer is stabilized by covalent crosslinking, the molecular mechanism by which such crosslinks are formed has not been revealed. Here we aimed to identify the enzyme(s) that crosslinks the 7S dodecamer and characterize its expression in the kidney glomerulus.

Methodology: To elucidate the type of crosslinks, we did pharmacological inhibition of matrix crosslinking enzymes. Lysyl oxidases expression in mouse embryonal PFHR-9 cells and glomeruli was investigated by mass spectrometry, immunoblotting and qPCR. We performed *in vitro* crosslinking and assays within the native insoluble collagen IV network. To demonstrate the presence of lysyl derived crosslinks we did experiments with 2.4-dinitrophenyl hydrazine. **Results:** Pharmacological inhibition of candidate ECM enzymes revealed that lysyl oxidase activity is required for crosslinking of 7S polypeptides. Among all lysyl oxidase family members, LOXL2 was identified as the isoform crosslinking collagen IV in PFHR-9 cells. Biochemical analyses revealed that LOXL2 readily promoted the formation of lysyl-derived crosslinks in the 7S dodecamer, but not in the NC1 domain. We also established that LOXL2 is the main lysyl oxidase family member present in the glomerular ECM.

Conclusions: We demonstrate that LOXL2 is a novel component of the molecular machinery that forms crosslinked collagen IV networks, which are essential for glomerular basement membrane stability and molecular ultrafiltration function.

(Sponsored by To All Members Of Vanacore, Hudson And Proteomics Laboratories, Vanderbilt University Medical Center.)

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Generating mammalian prions with internal deletions.

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Introduction: Prions are PrP proteins with altered structures causing transmissible fatal neurodegenerative diseases. They are able to self-perpetuate through the formation of beta-sheet-rich assemblies that induce the structural conversion of normal globular PrP, however the mechanistic insights of this prion structural conversion remain elusive.

Methodology: To determine regions that might be relevant and which residues are required for conversion, we performed deletions in the globular domain of ovine PrP.

Results: Deletions of either four or five residues in the helix 2 of the globular domain essentially preserved the overall PrP structure and mutant PrP expressed in RK13 cells were efficiently converted into bona fide prions upon challenge by three different strains assayed. Prions with an internal deletion were self-propagating and de novo infectious for naïve homologous and wild-type PrP expressing cells. Even more, they caused transmissible spongiform encephalopathies in mice, with similar biochemical signatures and neuropathologies than original strains.

Conclusions: We show that convertibility of PrP is preserved despite shortening an alpha-helix and that several residues are not required to generate prion entities that are still able to convert the wild-type PrP and to transfer the strain-specific information. We thus identified a piece of the prion domain that is neither necessary for the conformational transition of PrPC nor for the formation of a stable prion structure. These findings provide new insights into sequence/ structure/infectivity relationship for prions.

BRCA1 protein expression is downregulated by miR-185, miR-93 and miR-107 in breast cancer tumors

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BRCA1 is a tumor suppressor gene which mutations confer a high risk to breast cancer. Fifty percent of hereditary breast cancer tumors without germline mutation have impaired BRCA1 expression, as well as 30% of sporadic cases. Somatic events that silence BRCA1 expression have been described in breast cancer tumors, such as promoter hypermethylation and BRCA1 gene deletion, however these mechanisms do not completely explain the loss of BRCA1 expression in breast tumors. Our aim is to identify miRNAs differentially expressed in tumors with negative or moderate BRCA1 protein, which could regulate BRCA1 expression. Total RNA from 50 fresh-frozen breast cancer tumors was isolated and miRNA profiling was performed using miRNA microarrays. Sixteen miRNAs were found exclusively upregulated in BRCA1-negative, and ten in BRCA1-moderate tumors. In silico analyses predicted eleven miRNAs that could regulate BRCA1 expression. The effect of five of these microRNAs was assessed using a luciferase reporter assay in HEK293T cells, where the complete BRCA1 3'UTR, or a segment of its coding region, was cloned within the 3' end of the Luciferase gene. Cells were co-transfected with each of the selected microRNAs. MiR-185, miR-93 and miR-107 significantly downregulated luciferase activity by binding to the 3'UTR. Also, we transfected these microRNAs into a non-tumorigenic breast cell line (MCF10A), where miR-93 diminished endogenous BRCA1 expression in a 48%, miR-185 in 39% and miR-107 in 38%, in relation to non-transfected cells. These results strongly support that these microRNAs are potential regulators of BRCA1 expression in breast cancer tumors. FONDECYT1120200, CONICYT21120269.

Regulation of mitochondrial function in muscle cells: inter-organelle communication and organelle abundance.

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Mitochondrial activity is of vital importance in almost all cellular types. The mechanisms underlying its regulation include mitochondrial dynamics, changes in substrate usage and efficiency, among others. As the "social" organelle it is, mitochondria are able to communicate with the endoplasmic reticulum (ER) to modify its metabolic function. Increased Ca^{2+} transfer from the ER to mitochondria stimulates Krebs cycle activity, leading to higher mitochondrial activity. Cellular mitochondrial function is also intimately associated to the content of the organelle. This parameter is regulated through the coordination of the opposing processes of mitochondrial biogenesis and mitophagy. We have shown that functional coupling between the ER and mitochondria exists in vascular smooth muscle cells (VSMC) and can be increased in response to external stimulus. Furthermore, increased mitochondrial activity is associated with a more quiescent and functional cellular phenotype. As for the regulation of mitochondrial content, we have found that obesity leads to a biphasic increment of mitochondrial markers in skeletal muscle. This response is paralleled with a time-specific modulation of autophagy. Our data show that in VSMC and skeletal muscle cells, mitochondria are flexible organelles, whose function and abundance can be modified to cope with energetics needs.

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Session Posters 1

1) Structural studies of Octarellin mutants: a Crystallographic approach.

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Octarellins are artificial proteins of more than 200 amino acids, designed *de novo* to adopt the $(\beta\alpha)_8$ fold or best known as TIM-barrel. Through directed evolution, Octarellin V could be optimized increasing it solubility and stability. This new version, named Octarellin V.1, displayed 16 mutations in comparison to the parental protein, and 4 of them are proline. Statistical studies demonstrated the unexpected probability to obtain these 4 prolines. Then, we postulate that these prolines are the key in the improvement of Octarellin V. We hypothesize that the prolines could be promoting a different folding pathway or just making more rigid the protein, but without altering the tertiary structure of Octarellin. To answer the hypothesis, the 4 prolines were mutated from Octarrelin V,1 and in parallel introduced into Octarellin V, with the goal to obtain the crystallographic structure of these 2 mutants and compare them with the reported structure of the Octarellin V.1. Both mutants were produced and purified, to then create stable and pure complexes with the crystallization helpers αRep and Nanobody. The complexes were assayed in vapor diffusion (hanging drop) and counter diffusion crystallization techniques. At this stage, we are waiting to obtain crystals of good quality to be diffracted in a synchrotron facility. However, because the high specificity of αRep and nanobodies to recognize their partners as structure discriminants, we can already conclude that these mutants adopt the same fold displayed by Octarellin V.1 and we only need the crystallographic confirmation.

3) Characterization of the SUMO modification of the transcription factor TFEB.

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The transcription factor EB (TFEB) is a key regulator of lysosomal biogenesis and it also coordinated expression of autophagy and lysosomal genes. TFEB target genes carried a DNA sequence in the promoters called CLEAR, for Coordinated Lysosomal Expression and Regulation motif. The CLEAR element overlapped with the DNA target site for the MiTF and TFE3 trancription factors also members of the MiT family of transcription factors. Interestingly, TFEB and the other MiT family members MiTF and TFE3 have been reported to be modified by SUMO1. SUMO belongs to the Ubiquitin-like family (UBls) of post-translation modifications. Covalent conjugation by SUMO. In humans, three functional SUMO proteins are found SUMO1, SUMO2 and SUMO3. All SUMO proteins are encoded as inactive precursors containing a C-terminal extension incapable to enter into the activation and conjugation cascade. The initial step for the activation of SUMO is the excision of the C-terminal extension by SUMO specific proteases that occurs directly after translate. In the particular case of SUMO the E1 activating enzyme is a heterodimer of the SUMO Activating Enzyme Subunits 1 and 2, Sae1 and Sae2, respectively. The cascade has only one E2, Ubc9. SUMO modification has been described in a few thousand substrates participating in fundamental processes regarding the identity, the ontogeny and the stress response ability in eukaryotic cells. Here, we characterise the SUMO modification of TFEB and additionally using CRISPR/Cas9 technology we described a new role for SUMO as a modulator on TFEB functions.

FONDECYT 11150532

5) Reengineering of TLR2 modulating peptides from the human microbiota as new drugs for metabolic diseases.

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Introduction: Toll-like receptors (TLRs) are proteins that recognize structural patterns from microbe-derived molecules, as well as endogenous signals. To date, many studies have related TLR2 to several metabolic diseases. Peptide motifs from both endogenous and exogenous proteins from the human microbiota have been associated with the activation/ inhibition of TLR2 under different conditions or diseases. In this work, we identified conserved motifs from natural modulators of TLR2 from the gut microbiota, and re-engineered them to generate new potential peptide drugs for metabolic diseases.

Material and Methods: Sequence similarity networks were built to identify conserved regions of the TLR superfamily, especially the TLR2 family. Global and local alignments were performed to generate a library of conserved motifs from natural TLR2 modulators from the human microbiota. The library was then subjected to *in silico* site-specific mutations, and the resulting peptides were evaluated for their binding energy for TLR2 using molecular docking and molecular dynamics.

Results: We identified conserved peptide motifs from natural TLR2 modulators associated with metabolic diseases. In addition, through re-engineering, we have created a library of new possible TLR2 modulating peptides, which are potential candidate drugs for metabolic and inflammatory diseases.

Discussion: Many natural TLR2 modulators from the human microbiota play a key role in the immune system and metabolic diseases. Our preliminary results show that re-engineered peptides can bind with higher affinity energy than natural modulators, suggesting that they can be used as novel peptide drugs.

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7) New method for microsome preparation from Saccharomyces cerevisiae for the study of the mechanochemical mechanism of BiP.

Alfaro-Valdés H M[1], Retamales E[1], Lesch R[2], Wilson C A M[1], [1]Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.[2]Department of Molecular and Cellular Biology, Howard Hughes Medical Institute, University of California. (Sponsored by FONDECYT 11130263 PCI-PII20150073)

Posttranslational translocation (PT) of proteins through the endoplasmic reticulum (ER) is mediated by both a channel protein called Sec61 and auxiliary motor proteins called BiP. BiP is a member of the family of ATPase Hsp70 type chaperones, and studies suggest that it would be involved in the application of force during the PT of the polypeptide. The work exerted by BiP in the translocation of protein is not clear; however studies suggest that BiP could be involved in a passive or active mechanism 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. This study shows a new method of obtaining pure microsomes based on the disruption of the cells by glassbeads followed by a sucrose gradient. We obtained a yield of purified microsomes of 1.84 µg/L of culture of *Sacharomyces cerevisiae,* while we obtained 1.92 µg/L of culture using a conventional method (SM). The new method reduces costs and time of production; however the microsomes have a lower efficiency of translocation than those prepared by SM. This method can be improved by adding 1mM of BiP during lysis and can also be used to test mutant forms of BiP. These microsomes will be used to determine the mechanochemical properties of BiP by assays in bulk using a chimera protein that contains the signal sequence recognized by Sec61 and a protein mutated to remain unfolded (Titin) linked to Calmodulin (CaM). In this work we will show our progress in designing the CaM chimera.

9)Funcional relationship between NUAK1 and the SALL2 transcription factor under metabolic stress.

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Metabolic reprogramming of cancer cells is critical for survival under metabolic stress and subsequent invasion and metastasis. NUAK1, a member of the AMPK related kinase (ARK) family plays a pro-survival role under metabolic stress and is found deregulated in several cancers. NUAK1 overexpression promotes cell survival, migration and metastasis; however, how NUAK1 is regulated is largely unknown. Transcriptionally, only the large-MAF family members c-MAF and MAFB transcription factors are known to interact with *NUAK1* promoter, nevertheless, neither c-MAF nor MAFB responds to metabolic stress. Previous results suggested that SALL2 transcription factor is involved in cell survival under metabolic stress. Here, we investigated NUAK1 expression under metabolic stress and its possible regulation by SALL2. By qPCR and Western blot we found a positive correlation between the increase of SALL2 and NUAK1 levels under metabolic stress conditions, including serum and glucose deprivation. Consistent with a positive correlation between SALL2 and NUAK1 expression, *Sall2*$^{-/-}$ mouse embryonic fibroblasts (MEFs) showed a significant decrease on NUAK1 mRNA and protein levels in comparison with *Sall2*$^{+/+}$ MEFs. Additionally, loss of function experiments demonstrated that both, SALL2 and NUAK1 are required for the survival of cells to metabolic stress. Bioinformatics analysis of *NUAK1* promoter identified several putative SALL2 binding sites. We cloned the *NUAK1* promoter and performed reporter assays, finding that SALL2 increases *NUAK1* promoter activity. Taken together, our data suggest that SALL2 transcriptionally regulates NUAK1 expression, which relates to the survival of cells under metabolic stress. We are further investigating the mechanism by which SALL2 regulates NUAK1.

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11) Multidimensional free energy calculations of veratryl alcohol adsorption to P. chrysosporium lignin peroxidase.

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Lignin peroxidase (LiP) is a class II fungal heme peroxidase that catalyzes the oxidation of high redox-potential (non-) phenolic aromatic compounds such as veratryl alcohol (VA), which is a metabolite produced by *P. chrysosporium*. LiP is of high biotechnological interest due to its key role in lignin degradation to plant biomass (Hammel-et-al., 2009). The catalytic cycle of LiP involves the transfer of a radical to a solvent-exposed tryptophan, Trp^{171}, which can then oxidize VA to a radical cation species ($VA^{\bullet+}$) (Smith-et-al., 2009; Sáez-Jiménez et al., 2016); the latter has been found to act as a redox mediator in the oxidation of the bulky lignin polymer (Goodwin-et-al., 1995; Khindaria-et-al., 1996). Consequently, VA diffusion and recognition at the peroxidase protein surface are critical events during the enzymatic process, albeit few investigations have addressed them. We recently explored the binding and stabilization of VA onto the LiP surface through molecular dynamics and MM/GBSA calculations (Recabarren et al. 2016). A hydrophobic concave ditch, next to Trp^{171}, was observed to also stabilize VA, which could be considered as a transient site. Here, we present a three-dimensional free energy (ΔG) surface of VA and $VA^{\bullet+}$ adsorption onto the LiP WT surface (and three of its mutants), calculated using the adaptive biasing force (ABF) method. We identified energy minima at previously reported binding sites, and provide molecular characterization of the most stable VA conformations. The calculated ΔG values can be associated with affinities of VA in different states. This protocol would be extended to other LiP substrates.

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13) IDENTIFICATION AND EXPRESSION PROFILES OF LONG NON-CODING RNAs IN THE CHILEAN SEA URCHIN (loxechinus albus).

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The red sea urchin (*Loxechinus Albus*) is an endemic echinoderm species distributed along the Chilean coasts, which has been recently included in diversification aquaculture programs (PDACH). Currently, there are a few sequences in database, restricting the molecular study in this species. Recently the long non-coding RNAs (lncRNAs) are being considered as a new target of study because they could be involved in many processes still unknown. In this work, we study the pattern expression of lncRNAs and transcriptional differences associated to three tissues in *L. albus*, using the next generation sequencing techniques.Total RNA was extracted from gonad, gut and coelomocytes of three sea urchin, the samples were sequenced in paired-end using Miseq technology (Illumina) in duplicate. Raw data were filtered using flexbar software, de novo assembly and differential expression pipeline was performed CLC Genomics Workbench. Finally, we identified lncRNAs with CPAT, CD-search tool and homology data base. Sequencing generated 95.745.640 reads of which 5% was filtered, leaving 91.119.300 reads. Assembly obtained 131.090 contigs, with a N50 of 1.089bp. Subsequently, were obtained 15.952 filtered putative lncRNAs and 8.785 coding RNA. We detected putative lncRNAs differentially expressed in the three tissues with specific patterns of expression, furthermore correlated with coding RNA. In conclusion, we generated the first analyisis of lncRNA expression in loxechinus albus, and identified correlation transcripts and their expression pattern in the three different tissues. Our work provides a transcriptomic resource for future gene expression analysis in this species.

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15) Study and characterization of novel anti-tumoral triterpenes extracted from Quillaja saponaria.

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Therapeutic agents derived from plant triterpenes have become in an important group of phytochemicals for the pharmaceutical industry, owing to its biological properties (anti-inflammatory, anti-bacterial, anti-tumor and adjuvant). Their structural diversity draws attentions, because could to exist a relation between its structure and the diverse biological activities of this class of molecules. Several studies have revealed that triterpenes have a great potential in anti-tumor therapies, because can to alter biological membranes, specifically cholesterol, and could to affect diverse cellular activities, related with regulation of the cell cycle, proliferation and apoptosis.

In the current work, new triterpenes molecules from *Quillaja saponaria* (Molina), were purified using HPLC to preparative scale and characterized the biologicals activity as: cytotoxicity of molecules by MTS assay and Annexin V by flow cytometry on gastric tumor cells (SNU1, KATOIII and AGS).

We first, purified two new molecules (SAP1 and SAP2) by HPLC and analyzed the anti-proliferative effect on gastric cancer cell lines at 24 and 48 hrs. Using MTS Assay both molecules exhibited increased inhibition of proliferation at micromolar concentrations at 48 hrs of treatment. Moreover, was observed a minor effect on GES1 cell line (normal gastric cells used as control). SAP2 presented the ability to induce apoptosis of cancer cells examined by Annexin V-FITC.

Further studies are required to evaluate death mechanism of this new molecules on gastric cancer cells. However, these results are exciting, because open a novel opportunity to treatment of gastric cancer, using natural compounds extracted from a native species of Chile.

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17) Cardiomyocyte-specific polycystin-1 deficiency contributes to myocardial ischemia/reperfusion injury: role in cell death and paracrine effects on fibroblast to myofibroblast differentiation.

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Introduction: Myocardial ischemia and reperfusion (I/R) results in cardiomyocytes death and ventricular remodeling. Polycystin-1 (PC1) is a cardiomyocyte expressed mechanosensor whose absence causes cardiac dysfunction. This work aims to assess the role of PC1 in cardiomyocytes cell death and fibrosis-related effects induced by myocardial I/R.

Methodology: PC1 knockout (KO) mice were subjected to *in vivo* or *ex vivo* myocardial I/R. Infarct size was assessed by TTC staining. PC1 knockdown neonatal rat ventricular myocytes (siPC1 NRVMs) were subjected to simulated I/R. Cell death was assessed by LDH activity and apoptosis was analyzed by immunodetection of caspases 3, 8, 9 and Bax/Bcl2 levels. Paracrine effects on fibroblast a-SMA expression were assessed using NRVMs conditioned media.

Results: PC1 KO mice subjected to I/R show a larger infarction size and more LDH activity than control group. Also, siPC1 NRVMs present higher sensitivity to I/R induced cell death, showing more LDH activity and higher levels of cleaved caspase-3. We found a significant activation of caspase-8 in siPC1 NRVMs after I/R, meanwhile caspase-9 and Bax/Bcl2 levels were not affected. Cardiac fibroblast shows impaired differentiation rate when using siPC1 NRVMs conditioned medium.

Conclusions: Here we show that PC1 deficiency increases I/R-induced cell death in three different models. PC1 actively participates in the survival of cardiomyocytes subjected to I/R, probably sensing the mechanical changes generated during I/R, and triggering signals that regulate cellular death processes such as necrosis and apoptosis. Moreover, PC1 emerges as a new key regulator of the fibrotic response and cardiac remodeling after I/R.

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19) Effect of drug-drug interaction on transcriptional expression of metabolizying enzymes and a drug resistance protein in diferent tissues of rainbow trout (Oncorhynchus mykiss).

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Introduction: Chilean salmonid farming has been exposed for decades to different kinds of diseases, causing high mortality and considerable economic loss to the industry. In order to control different pathogens many drugs are consecutively used, such as Emamectin Benzoate (EMB), Oxytetracycline (OXY), Florfenicol (FF) and Erythromycin (ERY). The aim of this study was to evaluate the effect of these treatments on transcriptional expression of the metabolizing enzymes CYP2K1, CYP2M1, CYP3A27, FMO, GST and the drug resistance protein, Pgp, after single treatments with EMB, antibiotics and EMB/antibiotic consecutive treatments.

Material and Methods: Two independent experiments were carried out. In the first one, rainbow trout were independently administered with EMB, OXY, FF and ERY through feed. In the second experiment, rainbow trout were administered with EMB and then separately coadministered OXY, FF and ERY through feed. The transcriptional expression was determined by RT-qPCR.

Results: EMB increased the transcriptional expression of metabolizing enzymes, mainly in kidney, which was altered after the consecutive EMB/antibiotic treatments. Although EMB had no effect on the Pgp in any tissue, the consecutive EMB/antibiotic treatments increased its transcriptional expression.

Discussion: The transcriptional effect of single treatments with EMB and antibiotics was very different to EMB/antibiotic consecutive treatments, evenon the same tissue, suggesting the existence of a mechanism of pharmacological interaction between EMB and the antibiotics tested. Thus, treatment schedules between antibiotics and antiparasitic drugs, as well as, the possible pharmacological interactions should be more deeply reviewed.

Funded by: FONDECYT 1150934 and FONDAP 15110027. Dirección de Investigación y Desarrollo, Universidad Austral de Chile.

21) Overexpression of trnaglyucc affects growth of schizosaccharomyces pombe.

Arias L[1], Moreira S[1], Orellana O[1], [1]Biología Molecular y Celular, ICBM, Facultad de Medicina, Universidad de Chile.

Under stress conditions, tRNAs can be affected by changes in their levels as well as chemical modifications that might alter their function, including translation efficiency. Several tRNAs from *Saccharomyces cerevisiae*, including $tRNA^{Gly}$, are induced under oxidative stress. It is not known whether this induction affects protein translation. The purpose of this work was to determine whether increasing the tRNA levels affects the expression of proteins and/or the cell growth of *S. pombe.* We used H_2O_2 to induce oxidative stress condition in culture and we analyzed tRNA levels by northern blot. Our results showed that in *S. pombe* under H_2O_2-induced oxidative stress, $tRNA^{Gly}_{UCC}$ and $tRNA^{Arg}_{UCU}$ increased their levels while $tRNA^{Thr}_{UGU}$ did not change. Because $tRNA^{Gly}_{UCC}$ has a low gene dosage and decodes a non-optimal codon, we studied the effect of the overexpression on this tRNA in cell growth and shape. Overexpression of $tRNA^{Gly}_{UCC}$ gene in *S. pombe* slowed down growth and the cells showed an elongated shape. We hypothesize that these physiological changes could result from the alteration of either the expression or folding of cell cycle proteins such as cdc13, (the gene encoding cdc13 possesses GGA codons recognized by the $tRNA^{Gly}_{UCC}$). In conclusion, these results showed that under stress conditions the increase of tRNA levels in *S. pombe* occurs in a species-specific manner and suggest that it might affect the cell growth and fitness.

FONDECYT Regular 1150834 FONDECYT Postdoctorado 3150366 Beca Conicyt Magister nacional 22151224.

23) Targeting the oligomerization of fructose-1,6-bisphosphatase as a potential therapeutic approach for treatment of type 2 diabetes mellitus

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Overproduction of glucose via gluconeogenesis is a principal cause of the high blood glucose levels found in patients with type 2 diabetes, and is inadequately controlled by currently available medications. Fructose-1,6-bisphosphatase (FBPase) catalyzes the hydrolysis of fructose-1,6-bisphosphate and a divalent metal ion is required. It is a tetramer with identical 37 kDa subunits organized as a dimer of dimers (C1C2 and C3C4); the enzyme is active only in its tetrameric state. Due to its strategic position in the gluconeogenic pathway, hepatic FBPase has become an interesting target for development of new anti-diabetic pharmaceuticals. Until now the efforts have been focused on the use of AMP analogs, but peptides are likely to be more suitable as drugs than analogous of AMP, because they are expected to be more specific. Thus, a compound mimicking the properties of one of the interfaces should act as a competitive inhibitor and prevent interaction between the subunits. We have assessed the effect of various peptides homologous to the full or partial region of the C1C4 interface of FBPase (α-helices H1−H3) on its quaternary structure and enzymatic activity. The synthetic peptide like alfa-helix H3 (amino acids 73-90) inhibits FBPase activity (30%) and prevents its free subunit exchange, phenomenon that can be due to the interaction of the peptide with the interface C1-C4; docking analysis support this idea. The mechanism of inhibition by this peptide is not yet elucidated but this peptide could provide a promising strategy to lower glucose levels and treat type 2 diabetes mellitus.

FONDECYT 1141033 DID UACh 2013 45.

25) Characterization of Microsorum scolopendria obtained in Easter Island for a possible therapeutic use.

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Microsorum scolopendria is a wild growth fern on Easter Island. In alternative medicine it is used for different purposes, being part of some treatments in diseases such as breast cancer. Coumarin (1,2-benzopyrone) is one of the active compounds that gives the biological activity to the species. Studies show that coumarin has anti-inflammatory and antibacterial activity.

Methods: Samples of ecotypes of the plant are concentrated obtaining extracts rich in polyphenols Using this extracts of plants collected in Easter Island, antioxidant activity assays are performed using the DPPH reagent at a fixed concentration (0.05 mM) and different concentrations of extract (500-3000 µg/mL). To assess the viability and cytotoxicity of extracts MTS assay was performed on the cell line MCF-7.

Results: After tests conducted were obtained IC50 of DPPH assay and viability and cytotoxicity of various extracts of different ecotypes of *Microsorum scolopendria* in cell line MCF-7.

27) **Characterization of T lymphocyte population in the thymus of the rainbow trout.**

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The thymus in vertebrates plays an important role in producing functionally competent T-lymphocytes. In teleost fishes, thymus is a bilateral organ represented by thin oval laminae of lymphoid tissue, placed subcutaneously at the dorsal commissure of the operculum. In some teleost fishes such as the rainbow trout, the thymus is not fully described. In order to identify the thymus in the rainbow trout and to characterize the thymocytes, we submited fish to a period of 16 h of light and 8 h of darkness to allow thymus development. After a month of treatment, the thymus were extracted and a histological study of the organ was carried out. Clustered lymphocytic populations and Hassall´s corpuscles characteristic of this organ were observed. Moreover, the cell populations studied by means of flow cytometry. Using anti-CD4-1 and anti-CD8a antibodies, simple and double positive T cells were identified within the gate of lymphoid cells. The main population was $CD4^+$ $CD8^+$ cells accounting for 82,7% of total lymphocytes. $CD4^+$ $CD8^-$ cells were 10,6% and $CD4^-CD8^+$ cells were 1,2%. Using the same antibodies, we analyzed the thymus by confocal microscopy and observed the cell distribution of the double and single positive thymocytes. Subsequently, sorting T cells was performed to separate $CD4^+CD8^-$ and $CD4^+CD8^+$ cells. RT-PCR analysis showed the presence of TCRα, TCRβ and RAG1 transcripts in these cells indicating that they were actually thymocytes. The expression of RAG1 gene suggests that these cells have active recombination processes as expected T cells under development in the thymus.

RCUK MR-NO2526X-1.

29) Pro-inflammatory response in skin and mucus of Atlantic salmon (Salmo salar) and Coho salmon (Oncorhynchus kisutch) during infestation with Caligus rogercresseyi.

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Introduction. Skin and epidermal mucus are important components of the innate immune system in fish being cytokines the key mediators of this response. The present study was carried out to compare the pro-inflammatory response to infestation with *Caligus rogercresseyi* (Boxshall and Bravo 2000) in skin and epidermal mucus of Atlantic salmon, susceptible specie and Coho salmon, a specie resistant to infestation by this parasite.

Materials and Methods. Skin and epidermal mucus samples were obtained from juvenile of *S. salar* and *O. kisutch* during days 1, 11 and 19 post-infestation with caligus, corresponding to different stages of development of this parasite (copepodid, chalimus and adults). The expression levels of cytokines in skin and mucus were evaluated by Western blot and ELISA.

Results. *S. salar* and *O. kisutch* showed variations related to both, susceptibility and the expression levels of pro-inflammatory cytokines. Variations on the cytokines expression according to both, the stages of caligus development and the species of salmonids were also observed. **Conclusion.** This is the first study linking detection and protein quantification to a pro-inflammatory response between a susceptible *vs*. a resistant host to the infestation by caligus, clarifying a little bit more the mechanisms associated with differential susceptibility/resistance between this two species of salmonids.

Funded by: FONDECYT 1150934 and FONDAP 15110027 from CONICYT-CHILE. INNOVA CORFO 14IDL2-30112. Dirección de Investigación y Desarrollo (DID-UACh), Universidad Austral de Chile.

31) Bioinformatic tools for determination of high-affinity peptides as alternative to treatment of obesity.

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Introduction: Opsonin C3b is an important part of the immune system, being able to trigger phagocytosis by binding to the complement receptors (CR1) of macrophages. The coating of cells with C3b positions them at risk of destruction[1]. The FABP4 and AQP7 proteins are highly specific in adipocytes[2,3], therefore they can be targets for the generation of dual agonist of adipose cells and CR1, through hybrid peptides for the activation of the phagocytosis. In this work we will perform a bioinformatic analysis through the design of peptides, based on these sequences to trigger phagocytosis of adipocytes as an alternative to obesity treatment.

Materials and Methods: For the development of this work, it was used Uniprot, NCBI PROTEIN BLAST, and PDBsum as databases, Patchdock and FireDock for the realization of the Docking and I-Tasser for modeling of the AQP7 protein. All of them of open access.

Results:
Fig 1: Modeling through I-Tasser AQP7.
Fig 2: Interaction between C3b and CR1 for the determination of agonist peptides.
Fig 3: Interaction between FABP4 and IgG for the determination of FABP4 agonist peptides.

Conclutions: From the interaction sites between C3b and CR1, it was possible to obtain four agonist receptor peptides. From the modeling and subsequent docking between AQP7 and other protein structures (IgG) were found six binding peptides. Finally the analysis between FABP4 and other protein structures reported (PDB: 5C0N) generated 2 binding peptides. For the design of the hybrid peptides, a matrix was generated with 32 resulting peptides capable of binding to CR1 to AQP7 and FABP4.

References: [1]Gros P, et al, 2008 . [2]Furuhashi M. et al., 2008. [3]Matsumura K. et al., 2007

33) Effect of sodium tungstate on intracellular reactive oxygen species in hk2 cells.

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The progression of diabetic nephropathy is attributed to the generation of reactive oxygen species (ROS). It is for this reason that numerous strategies have been developed aimed at interrupting the production of ROS at the intracellular level. In particular, it has been reported that Na_2WO_4 has a normoglycemic effect. In addition, our results indicate a decrease in renal damage in diabetic animals treated with Na_2WO_4. Based on the above, the effect of Na_2WO_4 on ROS will be evaluated in HK-2 cells.

Methods: The effect of Na_2WO_4 on the generation of intracellular reactive oxygen species in HK-2 cells, using the DCFH-DA and MitoSOX probes, was evaluated in the presence of high glucose and H_2O_2 inducers. In addition, the effect of Na_2WO_4 on antioxidant enzymes, measuring the gene expression of SOD 1, SOD2 and CAT, was evaluated.

Results: Na_2WO_4 has an effect on ROS, inducing the expression of antioxidant enzymes.

Conclusions: Na_2WO_4 could be a good candidate for the reversal of renal damage observed in diabetic nephropathy by having an effect on ROS.

Innova Corfo 13IDL2-235020

35) Computational assessment of key factors enabling the high plastic-degrading efficiency of the novel enzyme PETase.

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PET (poly-ethyleneterephtalate) is one of the most consumed synthetic polymers globally with an annual production of 40 million tons. Unfortunately, its non-biodegradability and cost-inefficient chemical recycling causes its accumulation in landfills and natural habitats, resulting in both waste-management and environmental issues. In fact, its rate of deposition in marine environments is larger than its rate of production, with 25,000 tons of plastic accumulated in the Chilean sea by 2010. Strikingly, some hydrolases of fungal and bacterial origin were found to have PET-degrading properties. Recently, a new cutinase that could efficiently degrade PET at room temperature was identified and termed PETase. This enzyme has no solved structure and its sequence identity to other PET-degrading cutinases is ~40%. In this work, we used the Rosetta3 suite to generate a model of PETase through a multiple template homology modeling approach and the Amber16 software for molecular dynamics on PETase and two thermophilic plastic-degrading cutinases (TfCut and LCC), revealing significant differences in backbone flexibility near the active site of PETase in comparison to its thermophilic counterparts, explaining its activity at room temperature. Representative clusters from these simulations were collected for docking studies with a model substrate (2PET) using the Rosetta3 suite, revealing key differences to other plastic-degrading enzymes, such as a highly accessible hydrophobic binding site that, although sharing multiple residues with other enzymes, binds 2PET with higher affinity and within a distinct geometrical framework. These results constitute a framework for future rational design efforts aiming to further increase the efficiency of PETase.

INACH RG_47_16.

37) Characterization of the Domain Swapping dimer of the cold shock protein from Bacillus caldolyticus using Optical Tweezers.

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Domain Swapping (DS) is a mechanism in which monomers exchange structural elements to form oligomers. Therefore, the monomers need to unfold total or partially to associate. Consequently, DS is favored at high monomer concentration and low stability conditions. In these conditions oligomer formation competes with aggregation making difficult the study of DS process. To avoid this complications we study the DS mechanism of the cold shock protein from B.caldolyticus (BcCSP) at single molecule level by using optical tweezers. To do this, we generated a fusion protein joining two BcCSP chains (henceforth BcCSPsc) and their folding mechanism was characterized pulling from its N and C terminal ends at constant velocity. Mechanical unfolding/refolding cycles of BcCSPsc showed two behaviors: 1) two sequential unfolding transitions followed by two sequential refolding transitions, and 2) one unfolding transition followed by one refolding transition. For the first case, the distribution of molecular extensions (the contour length, Lc) is unimodal with an average value of Lc expected for the independent unfolding of each domain of BcCSPsc. Nevertheless, in the second case, the distribution of molecular extensions shows an additional maximum that correspond to the complete and cooperative unfolding of BcCSPsc. This behavior is expected to occur when both domains of BcCSPsc acquire a DS configuration. The probability of occurrence of DS in BcCSPsc is about 1 % since most unfolding events are sequential. Therefore, our results suggest that BcCSPsc can adopt the structure of the domain-swapped dimer, although this configuration is energetically unfavorable.

FONDECYT 1151274, FONDECYT 1130510, Anillo ACT-1107, CONICYT 21150966, CONICYT 21130254.

39) Differential regulation of cardiomyocyte autophagy by angiotensin II and angiotensin-(1-9).

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Introduction: Angiotensin-(1-9), a new component of the non-canonical renin-angiotensin system, counteracts the cardiovascular actions by acting through the AT2 receptor (AT2R). Angiotensin II increases autophagy flux in vitro and in vivo models of pathological cardiac hypertrophy. Keeping autophagy in a homeostatic range is a key feature for any intended cardiac therapy. Here, our aim was to investigate if angiotensin-(1-9) modulates cardiomyocyte autophagy stimulated by angiotensin II.

Methods: Cultured neonatal rat cardiomyocytes were treated with 10 µM angiotensin-(1-9) for 6 h after exposure to angiotensin II (10 nM) for 24 h. Autophagic flux was assessed in cells incubated with chloroquine. Autophagy was evaluated by Western blot, monitoring the protein levels of LC3-II, Beclin1, p62 and GAPDH. The mRNAs levels of LC3b, Beclin1, Atg5, Atg12 and Gabarapl1 were determined by qPCR.

Results: Angiotensin-(1-9) modestly but effectively inhibited basal autophagy in cardiomyocytes after 6 h incubation. The pretreatment with angiotensin II stimulated cardiomyocyte autophagy, being prevented by angiotensin-(1-9). This peptide also restored to basal levels the expression of genes associated with autophagy induced by angiotensin II.

Conclusions: Angiotensin-(1-9), a natural peptide with anti-hypertensive, anti-hypertrophic and anti-remodeling effects, prevented the activation of autophagy triggered by angiotensin II.

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41) Herpud1 regulates insulin response in skeletal muscle cells

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Introduction: Herpud1, a protein located at ER, is induced by different stressors. A recent work showed that Herpud1 knockout mice are intolerant to glucose challenge, without changes on insulin secretion. Considering that skeletal muscle is the main responsible for insulin-dependent glucose uptake, our aim was to investigate if Herpud1 is required for normal insulin response in skeletal muscle cells.

Methods: L6 rat-derived skeletal muscle cells differentiated into myotubes were used as cellular model. GLUT4 translocation to plasma membrane was evaluated through OPD assay; glucose uptake was determined by 2-NBDG assay; protein levels were determined by Western blot; Herpud1 was depleted using a specific siRNA. Plasmids (Herpud1, Herpud1-GFP, GFP and pcDNA3.1) were transfected using Lipofectamine 2000 and cells were infected with adenovirus (MOI: 1.000) for 48 h.

Results: Herpud1 knockdown decreases insulin-triggered Akt^{Ser473} phosphorylation, GLUT4 translocation to plasma membrane and glucose uptake in L6 myotubes. The overexpression of Herpud1 increases insulin-dependent Akt^{Ser473} phosphorylation and glucose uptake. Herpud1 depletion increases activity of Ser/Thr phosphatase calcineurin. Calcineurin regulates Akt phosphorylation. Both pharmacological and genetic inhibition of calcineurin restores insulin-induced Akt^{Ser473} phosphorylation and glucose uptake in Herpud1 depleted L6 myotubes.

Conclusion: Herpud1 is required for normal insulin-induced glucose uptake in skeletal muscle cells by modulating calcineurin activity.

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43) Exploring the intracellular pH-sensing mechanism of TASK-2 K2P channel Bustos Daniel, Sepúlveda Francisco, González Wendy

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Two-pore domain potassium (K_2P) channels are responsible for maintaining the background conductance essential to the resting membrane potential. K_2P channels assemble as dimers containing two pore-forming domains and four transmembrane segments per subunits. Two fenestrations connect the lipid membrane with the central conduction cavity, which can be open or closed depending of the movements of helix TM4. TALK subfamily of K_2P channels is activated by alkaline extracellular pH and is formed by 3 members: TALK-1, TALK-2 and TASK-2. TASK-2 is also gated by intracellular pH (pH_i), being closed by intracellular acidification and activated by increasing pH_i. The neutralization of lysine positioned at the end of TM4 helix, and probably within the fenestrations, by mutation to K245A abolishes pH_i-gating. The molecular mechanism by which pH_i-sensing K245 exerts its gating role is unknown. A possible mechanism by which pH_i-dependent protonation/deprotonation of K245 affects the permeation of K^+ ions is an electrostatic effect in the conduction pore. We propose to evaluate this hypothesis by studying the underlying free-energy profiles. We will employ the adaptive biasing force method to compute the free-energy profile in the conduction pore using TASK-2 homology models with the fenestrations open or closed. Analyses of these profiles with K245 residue in a protonated/neutral states will throw light on the plausibility of the hypothesis.

45) Effect of viral proteins of Infectious Pancreatic Necrosis Virus (IPNV) on the cellular mRNAs translation.

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Infectious pancreatic necrosis virus (IPNV) is a virus that infects salmonids, becoming of economic relevance on salmon farming. IPNV belongs to the family Birnaviridae, genus Aquabirnavirus. It is non-enveloped virus, with a diameter close to 60 nm and its genome is composed of two segments of uncapped and non-polyadenilated double-stranded RNA, segment A and B. The Segment A encodes for a small protein, VP5, and a polyprotein. The polyprotein is autoprocesed by viral protease VP4 to generate the capsid protein VP2, VP4 and the matrix protein VP3. The segment B encodes for VP1, the viral RNA polymerase RNA-dependent of 94 KDa, which can be found free or covalently attached to 5 ends of each segment, named Vpg. It has been shown that viral infection inhibits the synthesis of cellular proteins at the same time as the viral polypeptides begin to be detected. In order to identify the viral proteins responsible for the cellular translation inhibition, proteins VP1, VP2, VP3 and VP4 were cloned into plasmids to transfect fish cells CHSE/F, susceptibles to IPNV infection. The effect of viral proteins was evaluated on Cap-dependent and Cap-independent translation, using bicistronic reporters. The results show that VP1 and VP4 inhibit the Cap-dependent translation, while VP4 active Cap-independent translation, and the effect of VP1 on Cap-independent translation depends on its concentration on cells.

FONDECYT N°1150901

47) Transcriptional and protein expression in tail fat from Chilota and Suffolk Down lambs grazing Calafatal.

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This experiment was carried out to evaluate the breed effect on the transcriptional and protein expression of lipogenic key enzymes and one transcription factor in tail fat from 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 standards. Fatty acid profile, qRT-PCR and Western blotting analysis from tail fat were performed.mRNA expression levels of lipogenic enzymes did not significantly differ between both breeds of lambs (P>0.05). FAS and SREBP1 showed a trend of overexpressed in Chilota than Suffolk Down lambs (P > 0.05), however protein expression for ACC, FAS, SCD and SREBP1 was higher in S. Down than Chilota lambs (P<0.05). The sum SFA did not show any significant difference between breeds (P>0.05), although ACC, FAS and SREBP1 protein expression were higher in Chilota than S. Down lambs. n-*6* PUFA proportions were higher in Chilota than S.Down lambs grazing Calafatal not being consistent with SCD transcriptional (P>0.05) or protein expression. In summary, although transcriptional and protein expression results were contradictory, a lower *de novo* SFA synthesis and higher sum PUFA and *n-6* PUFA proportions were expected in Chilota than Suffolk Down lambs grazing Calafatal.

InnovaChile 14IDL2-30112. FONDECYT 1150934 and 3160059.

49) Gastric cancer derived extracellular vesicles containing miR-335-5p inhibit cell invasion.

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Introduction: MiR-335-5p has been reported to be dysregulated in various types of cancer. We have analyzed the expression of miR-335-5p in advanced gastric cancer (GC) tissues relative to their paired adjacent non-tumor tissues and validated that miR-335-5p is downregulated in advanced GC. We have also demonstrated that miR-335-5p overexpression correlates with a variety of biological processes in tumor cell.

Methods: EVs were isolated from supernatants from two GC cell lines (AGS and HS746T), cells transfected with miR-335-5p mimics and from plasma patients' samples and characterized by western blot and nanosight. Using qPCR the expression levels of miR-335-5p were analyzed in all samples including EVs. First, the invasive properties of both cell lines and cells transfected with miR-335-5p mimics were studied. Next, the effect of EVs derived from untreated cells and cells overexpressing miR-335-5p on the invasive activity of AGS and HS746T GC cell lines was investigated.

Results: In comparison to AGS cells, HS746T cells express significantly less miR-335-5p and demonstrate higher invasive properties. In accordance with these findings cells transfected with miR-335-5p mimics show significantly decreased invasive properties. MiR-335 is also expressed in the EVs derived from both GC cell lines and patients plasma. EVs isolated from AGS and HS746T vary in their effect on invasive properties. EVs derived from GC cells overexpressing miR-335-5p significantly suppress invasion in both GC cell types though the effect is more pronounced in HS746T cells.

Conclusion: MicroRNA-335-5p is expressed in GC derived extracellular vesicles and modulates the invasiveness of GC cells.

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51) Biochemical characterization of 4-amino-5-hydroxymethyl-2-methylpyrimidine kinase (HMPK) from Thermus thermophilus and Salmonella Typhimurium.

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In bacteria, 4-amino-5-hydroxymethyl-2-methylpyrimidine kinase (HMPK) is an essential enzyme of the thiamine biosynthetic pathway (vitamin B_1), catalyzing two consecutives ATP-dependent phosphorylations. The first of them is that of 4-amino-5-hydroxymethyl-2-methylpyrimidine (HMP) to produce HMP phosphate (HMP-P) as intermediary, and then HMP pyrophosphate (HMP-PP) as final product. Until now, HMPK from *Escherichia coli* (ThiD Gene) is the only bacterial enzyme kinetically characterized. Although the structures of HMPKs from *Thermus thermophilus* and *Salmonella Typhimurium* have been solved, no studies regarding their biochemical properties have been reported. Here, we provide the first characterization of these enzymes. We demonstrated trough stoichiometric and HPLC analyses that enzymes from *S. Typhimurium* and *T. thermophilus* are able to perform the two consecutive phosphorylation of HMP. Size exclusion chromatography experiments showed that these enzymes are monomers under the conditions evaluated, in contrast with the *E. coli* enzyme which has been reported as a tetramer. Also, steady-state kinetics and thermal and chemical stability parameters of both enzymes were determined. These results shed light on the common characteristics of these phylogenetically distant enzymes and may help in the development of new inhibitors of bacterial thiamine biosynthesis.

FONDECYT 3160332, 1150460 and 1170701

53) Loop assembly: Open source tool for community-based plant engineering.

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Several DNA assembly systems have been developed in the recent years for the routine assembly of genetic constructs increasing in complexity and size. One of the most important advances corresponds to new DNA fabrication techniques, such as the Golden Gate and one-step assembly methods, also known as “Gibson assembly”, which coupled to the reduced cost of DNA synthesis have greatly increased reliability and efficiency of DNA fabrication. Whilst each system employs a specific method, no DNA construction system which integrates cloning technologies and tackles the standardisation of DNA assembly in a generalised manner has been developed.

Here we present Loop assembly, a set of IP-free genetic resources and methods for multiplex, efficient and low-cost DNA fabrication that allows the assembly of up to 16 transcriptional units routinely from a broad range of basic elements (e.g. promoters, tags, repressors, etc). This method relies on the use of Golden Gate type IIs enzymes SapI and BsaI, plus T4 ligase, for 2h “one pot” reactions that uses 8 plasmids in two different levels for recursive DNA assembly. In addition, this system permits the shuffling of arbitrary combinations of previously assembled transcriptional units by using a set of standardized primers and flanking unique sequences for high efficiency Gibson Assembly.

This new hybrid system would allow scaling from very specific genetic elements obtained from repositories cured by the community through an openMTA license for unrestricted sharing and open access.

CONICYT-PAI/Apoyo al Retorno Folio 82130027, FONDECYT Iniciación 11140776. Millennium Nucleus for Plant Systems and Synthetic Biology, FONDAP Center for Genome Regulation Chile, UK Biotechnological and Biological Sciences Research Council (BBSRC), Synthetic Biology Research Centre ‘OpenPlant’ award (BB/L014130/1) and Beca Conicyt Doctorado Nacional 2015.

55) Role of ROS produced by mitochondria and NOX (NADPH-Oxidase) in apoptotic death of cerebellar granule neurons.

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Introduction: It has been described that reactive oxygen species (ROS) play a role in multiple processes during physiological and pathological conditions. There are several sources of ROS in the cell; recent lines of evidence show that exist an interplay between different sources, suggesting that ROS produced by the mitochondria induces the ROS production by NOX. NOX is a source involved in apoptotic neuronal death, however it is unknown if there is a crosstalk between ROS produced by NOX and those produced by the mitochondria. In cerebellar granule neurons (CGN) treated with staurosporine (ST) or potassium deprivation (K5), ROS production occurs at different times along of the apoptotic death process, but the fully mechanism is not well understood.

Methods: Cultured CGN was maintained in a medium with 25mM of potassium (K25) during 7 days *in vitro* (DIV) later, these neurons was treated with ST (0.5mM) or K5, under these conditions, we measure cytoplasmic or mitochondrial ROS production by using dihydroethidium or Mitotracker red CM-H_2XRos, respectively. To determine cell viability we used Calcein-propidium iodine stain and mitochondrial activity was estimated by MTT reduction.

Results: ST or K5 treatment induced a significant increase of cytoplasmic ROS after five hours, moreover an early increasing in mitochondrial ROS levels also was observed. On the other hand viability was not completely abolished with antioxidants treatment.

Conclusions: The results suggest that early mitochondrial ROS produced during this process participates in neuronal death process mediated by ST or K5 but is not the only involved, probably NOX also participates.

57)Transcriptomic analysis by RNA-Seq in a cellular model of aggregation of TDP-43 protein.

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Introduction: TDP-43 protein is implicated in RNA homeostasis and its aggregation is associated with neurodegenerative processes such as ALS and FTD. The relationship between TDP-43 aggregates and neurodegeneration are still unknown. An aggregation model of TDP-43 in HEK293 was used to provide relevant information about genes or metabolic pathways that are responding or being affected in a TDP-43 aggregation scenario.

Methodology: Three cell lines stably transfected were used to perform RNAseq: HEK293-Flp-in (control cell line), HEK293-TDP-43WT (TDP-43 WT overexpression) and HEK293-TDP-agg (overexpression of TDP-43 aggregated form). Obtained data was processed using Cufflinks v2.2.1 software. Various packages within the R software were used for statistical analyses. Functional annotation and metabolic pathways analysis were carried out using public database such as NCBI-nr, UniProt, Gene Ontology (Go) and KEGG. Hsc70 expression was assayed through qPCR as preliminary validation data.

Results: Comparison between each condition, TDP-43 WT overexpression and TDP-43 aggregation shows differential gene expression. Main changes were observed in pathways like protein chaperone responses, vesicle-mediated transport, protein processing in endoplasmic reticulum and autophagy. Among others, Hsc70 gene, an important component of Chaperone Mediated Autophagy (CMA), was up-regulated in TDP-43 aggregation condition. Up-regulation of Hsc70 after induction of TDP-43 aggregates was validated though qPCR assay.

Conclusions: Results in this work indicate that TDP-43 aggregation disturbs relevant pathways necessary for cell physiology maintenance. These changes may be responsible for the pathological mechanisms associated with TDP-43 aggregation in neurodegenerative diseases.

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59)Pharmacogenomic biomarkers for adverse reactions to chemotherapeutics in cancer patients. A Pre-Therapeutic Approach.

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Introduction: Adverse reactions to chemotherapeutic (ADR) agents are common in cancer treatment. Many of these are clinically manageable; however, others can seriously affect the patient´s life. In addition to clinical factors, patient´s genetic profile may be a major predictor of toxicity. Single nucleotide polymorphisms (SNPs) are now recognized as key factors in the variability of drug response. According to this, the purpose of this work is to find an association between pharmacogenetic markers and ADR in patients with gastric and ovarian cancer receiving platinum/taxane/ fluoropyrimidines drugs.

Methods: Blood and tumor tissues embedded in paraffin samples were obtained with informed consent from adult ovarian (n=120) and gastric (n=18) cancer patients. DNA was extracted and 35 SNPs in 22 genes were genotyped. High specificity Taqman probes were used.

Results: Four SNPs associated with risk of ADR within the ABC transporter family showed genotypic frequencies ranging between 0 - 23%. Frequencies for the ADR-associated variants in SNPs CYP 3A4/3A5/1B1 genes were between 4 - 90%. For the *CYP2C8* enzyme, whose function is essential in taxanes metabolism, frequencies of risk-associated SNPs ranged from 2.10% to 13.6%. Four risk SNPs in the *DPYD* gene, main metabolic enzyme of fluoropyrimidines, ranged from 1% to 27%.

Conclusions: Frequencies of SNPs associated ADRs in cancer chemotherapy are highly variable in the samples of ovarian/gastric cancer patients studied. Identification of these variants alone or in combination, together with the most relevant clinical parameters could be integrated through statistical techniques of data mining to generate predictive models.

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61) Changing provascular complexity may modulate iron content in Arabidopsis Seeds

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Introduction: Anemia due to iron deficit represents a major health problem worldwide. In most crops, iron levels are not sufficient to meet daily needs. Biofortification of seeds is an alternative to combat this problem, but little is known about the mechanism of transport and accumulation of iron in seeds. It has been described that iron accumulates in the endodermis cell layer, surrounding the provasculature in Arabidopsis embryos. Provasculature complexity is simple in Arabidopsis cotyledons, consisting in a primary central vein and secondary veins forming areoles. It has been described that changing levels of expression of some genes may modify provasculature complexity. For instance, overexpressing *OPS* produces embryos with increased vascular complexity; while *vcc ops* double mutants have reduced vascular complexity.We hypothesize that an increase in vascular complexity, raises the volume of the endodermis and, by consequence, the total iron content.

Methodology: Total iron content of seeds, dissected cotyledons and hypocotyls, were determined using ICP-MS. We evaluated vascular complexity in Arabidopsis embryos from different backgrounds using Perls/DAB stain. **Results:** Preliminary results show that two thirds of total iron in the Arabidopsis embryo is stored in cotyledons, and one-third is in hypocotyl. Preliminary results show that iron content is reduced in *vcc ops* double mutant.

Conclusion:A decrease in vascular complexity negatively affects iron content. This approach could be used as basis to develop strategies aimed to biofortify seeds.

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63) Truncation and functional analysis of two RNA aptamers, G70 and G37, targeted to the main toxin of Loxosceles laeta spider venom

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Loxoscelism, the envenomation caused by the bite of *Loxosceles* spiders, is characterized by dermonecrosis, haemolysis and even death. The venom contains several isoforms of a toxic sphingomyelinase D (SMD). There are no diagnostic means and treatment is mainly palliative: horse antisera, of unproven efficacy, elicit anaphylactic reactions. Aptamers obtained by iterative selection targeted to a recombinant Ll2, a *Loxosceles laeta* SMD isoform, may provide safe, specific and effective theranostic means. RNA aptamers G70 and G37, differing in one of 107 nucleotides, inhibit Ll2 sphingomyelinase activity 26 and 3%. G37 arose in multiple copies during selection, suggesting stronger binding. G70 and G37 were truncated in each of their three arms (A-B-C) of predicted secondary structure with the aims of making large scale chemical synthesis affordable (<60 nucleotides) and improving aptamer function. Aptamers were synthesized by *in vitro* transcription. Binding to Ll2 was measured using nitrocellulose filters and inhibition of the sphingomyelinase activity of three *Loxosceles laeta* SMD isoforms was determined fluorimetrically. Aptamer variants partially deleted in arm C (78 nt) retain binding to Ll2, G70 exerting greater inhibition on isoforms Ll1, Ll2, and LlPLD1 (30-26-10%) than G37 (8-3-0%). G70 lacking arm B (83 nt) supports less inhibition of Ll2 (18%) but retains inhibition of Ll1 (18%). Deletions of arm A are detrimental to G70 inhibition of Ll1 and Ll2. Overall, a 54 nt variant of G70 having arm A, but lacking arm B and part of arm C, may constitute the molecular basis of novel therapeutic and diagnostic agents for loxoscelism.

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65) The PI3K/ Akt pathway regulates the expression of MDR transporters in Glioblastoma Stem-like Cells.

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Introduction: Glioblastomas stem-like cells have been proposed as the main responsible for the high recurrence and chemoresistance of GBM. In these cells, the PI3K/Akt signaling cascade is activated and promotes ABC transporter-mediated Multiple Drug Resistance (MDR). Additionally, GSCs inhabit hypoxic niches which promote their stemness and MDR. However, the relationship between the hypoxic microenvironment and the role of PI3K/Akt signaling on GSC chemoresistance has not been studied. Therefore, the aim of this study was to evaluate the role of PI3K/Akt signaling on ABC transporters under conditions of normoxia and hypoxia in GSCs.

Methodology: The U87MG cell line was maintained for 7 days in a special medium, supplemented with growth factors for GSC enrichment. GSCs were then maintained for 24 hours in normoxia or hypoxia (0.1% O_2) and followed by incubation with 25 µM LY294002 (PI3K/Akt inhibitor) to evaluate ABC transporter expression and cell viability by Western blot and MTS, respectively.

Results: Analysis of the expression of the two most described transporters in GSCs: Mrp1 and MRP3, showed that the expression Mrp3, but not Mrp1, increased under hypoxia. In this same context, LY294002 decreases Mrp3 expression and increases Mrp1 expression. Finally, treatment with LY294002 decreased cell viability in both normoxia and hypoxia; however, we did not observe significant differences between the two conditions.

Conclusion: The PI3K/Akt pathway differentially induces the expression of Mrp1 and Mrp3 in U87 GSCs, depending on their microenvironment.

FONDECYT1160777.

67) Development of a novel catalytic amyloid displaying a metal- dependent ATPase-like activity

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Amyloids are protein aggregates of highly regular structure that are involved in diverse pathologies such as Alzheimer´s and Parkinson´s disease. Recent evidence has shown that under certain conditions, small peptides can self-assemble into amyloids that exhibit catalytic reactivity towards certain compounds. Here we report a novel peptide with a sequence derived from the active site of RNA polymerase that displays hydrolytic activity towards ATP. The catalytic reaction proceeds in the presence of the divalent metal manganese and the products are ADP and AMP. The kinetic data shows a substrate-dependent saturation of the activity with a maximum rate achieved at around 1 mM ATP. At higher ATP concentrations, we also observed substrate inhibition of the activity. The self-assembly of the peptide into amyloids is strictly metal-dependent and required for the catalysis. Our results show that aspartate-containing amyloids can also be catalysts under conditions that include interactions with metals. Moreover, we show for the first time an amyloid that exerts reactivity towards a biologically essential molecule.

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69) Role Of HERPUD1 In Osteoclast Differentation in vitro

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Introduction Bone remodeling is an active process for skeleton development and is essential for calcium homeostasis. Its integrity is through the coordination between osteoclast and osteoblast activities. Osteoclast maturation depends of differentiation and fusion of macrophages present in bone tissue, forming a polarized giant cell which secrete lysosomal hydrolases into the resorption area. This maturation depends of autophagy machinery. HERPUD1, an ER-membrane protein, is a key negative regulator of autophagy, but its role in osteoclastogenesis is unknown. The aim of our work is determinate the HERPUD1 function during osteoclast differentiation.

Experimental approach Pre-osteoclast RAW264.7 cells were treated with an inductor of osteoclastogenesis, RANKL, for 3, 7 or 14 days. To decrease herpud1 we used shRNA lentivirus, and we measured the expression of differentiation markers (Rank, mmp9 y trap) and *herpud1* using semiquantitative qPCR. To evaluate matrix resorption, we used Corning® osteoassay. Also, we standardized a new osteoclast activation assay, preparing bone matrix from bones ground purified from C57BL/6 mice legs. We plated osteoclasts and they were analyzed by immunofluorescence shown a characteristic cytoskeleton actin ring where they have contact with the bone matrix.

Results We observed an increase of osteoclastgenesis markers at 7 and 14 days post-stimulus and a reduction of HERPUD1 levels that correlate with an augment of autophagy markers. Silencing of expression of HERPUD1 increase of osteoclast differentiation phenotype, even without RANKL, siHerpud1 cells are TRAP positive.

Conclusion: These results shown that HERPUD1 has a negative role in osteoclastogenesis and could be a target to regulate bone remodeling.

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71) Incorporation of CPPs and their relationship to lipid composition in membrane models

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The plasma membrane maintains a stable composition according to the functions that it develops, in pathological situations this stability is deregulated. In diseases like Niemann-Pick drastically decreases cholesterol concentration while both phospholipids and cholesterol vary depending on the type and progression of cancer. Penetrant peptides (CPPs) arise as an alternative for facilitated and selective entry to these pathological situations. To imitate the cell membrane models are made with three constant lipids and cholesterol as variable lipid. To evaluate the selectivity of the input the perturbation of the hydrophilic region is determined by generalized polarization of Laurdan and of the hydrophobic region evaluating anisotropy of DPH. In order to compare and verify the entry of the synthesized peptides, the entry of rhodamine-labeled peptides was performed, allowing a quantitative analysis of this parameter.

73) The structural role of disulfide bridges of PETase, a Polyethylene terephthalate(PET) degrading enzyme.

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Recently, the novel bacterium *Ideonella sakaiensis* 201-F6 was described, which is able to degrade and assimilate polyethylene terephthalate (PET), and use it as its major energy and carbon source. This is achieved through a novel cutinase termed PETase which degrades this polymer into monohydroxyetilterephtalate, and MHETase which further breaks down such monomer into its nontoxic constituents.

So far, no structural cues about the high efficiency PET-degrading activity of this new enzyme have been reported . In this study we constructed a model of the PETase by comparative modeling with Rosetta3.6 using as template two closely related cutinases from *Thermobifida fusca* (Thf) and leaf branch cutinase (LCC),both of which are only capable of degrading PET at high temperatures.

Our results show that there is a energy difference of 70 REU between both HMs with(NAT) and without(DBBS) disulfide bridges, in which the NAT model had the lowest energy. Quantitative analysis using molecular dynamics(MD) simulations on both HMs using AMBER16 enabled us to further explore the effects of this disulfide bridge in the stability of its binding pocket. From these simulations, we determined that the root mean square fluctuation between both MDs in several regions near the active site increase, on average, 0.9 nanometers in the absence of the disulfide bond. Our results suggest this disulfide bond might be crucial for the function of this novel enzyme.

Proyecto INACH RG_47_16.

75) A2BAR regulates the migratory/invasive capacity of Glioblastoma Stem-like Cells under hypoxia.

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Introduction: Glioblastoma Multiforme (GBM) is the tumor with the highest incidence and mortality rates among neoplasms that affect the Central Nervous System. The high aggressiveness of GBM is due to the presence of a cellular sub-population known as Glioblastoma Stem-like Cells (GSCs). GSCs are responsible for the development of the GBM tumorigenic characteristics, emphasizing its high capacity to infiltrate healthy tissue; a limiting factor for total surgical resection. GSCs grow in a hypoxic microenvironment *in vivo*, which promotes increased extracellular adenosine (ADO) production, activating the low-affinity A_{2B} Adenosine Receptor (A_{2B}AR). The aim of this study was understand the role of A_{2B}AR in the migratory/invasive capacity of GSCs under hypoxia.

Methodology: GSCs derived from human GBM U87MG were cultured under normoxia and hypoxia (0,5% O_2) conditions. A_{2B}AR, MMP-2, and MMP-9 expression were measured by Western-Blot and ADO production by HPLC. To evaluate the migratory and invasive capacities of GSCs, we performed an adhesion/migration assay and Transwell Matrigel-Coated assay respectively. To evaluate the role of A_{2B}AR we used the selective antagonist MRS1754.

Results: GSCs under hypoxic conditions presented increased ADO production. Migratory and invasive capacities of GSCs increased under hypoxia. Use of the specific antagonist MRS1754 decreased the migratory/invasive capacity and, in addition, decreased the expression of MMP-2 and MMP-9 in GSCs under hypoxia.

Conclusions: Extracellular adenosine can regulate the migratory/invasive capacity and MMP expression in GSCs through the activation of the A_{2B}AR under hypoxic conditions

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77) Interaction between cyclic peptides Smac/DIABLO analogues and BIRC5 protein.

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Gastric cancer is one of the most common cancers in Chile. The identification of molecules that help human tumors growth and survival has been a molecular target for therapeutic strategies. BIRC5 is a member of the Inhibitor of Apoptosis Protein (IAP) family, which is overexpressed in most of cancers including gastric cancer, but is mostly absent in adult normal tissues. In this study we developed recombinant BIRC5 and synthesized cyclic peptides that contain the N-terminal tail of Smac/DIABLO to test their binding by steady state fluorescence anisotropy.

Five cyclic peptides were synthesized in solid phase with F-moc methodology. Cloning, expression and purification of recombinant BIRC5 was performed using AGS cell line from gastric cancer. Cell viability and cytotoxicity of peptides was measured with MTS assay on AGS, SNU1, KATO 3 and M1 cell lines. Binding of peptides to BIRC5 was measured by fluorescence anisotropy assay and visualization of cellular uptake of peptides with confocal microscopy.

Cyclic peptides are capable of penetrate membranes of all cell types we analyzed. Binding assays regarding purified recombinant BIRC5 and fluorescence labeled peptides showed low increases in fluorescence anisotropy. Cell viability was measured at different concentrations of peptides and there was no relevant difference between the control and treated samples.

Results indicate that cyclic peptides analogues do not bind to recombinant BIRC5 in vitro, given the fact that the anisotropy did not increase and the change in cell viability of treated cells compared to cells without treatment were almost the same in terms of percentages.

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79) Low cost and open source multi-fluorescence imaging system.

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The advent of easy-to-use microcontrollers, off-the-shelf electronics and customizable manufacturing technologies has facilitated the development of inexpensive open source scientific devices. We have created a low cost imaging system that integrates open-source hardware, software and wetware.

The hardware consists of readily available 470 nm LEDs, a Raspberry Pi camera and a set of filters made with low cost acrylics. This device allows imaging in scales ranging from single colonies to entire plates. We developed a battery of genetic components (e.g. promoters, coding sequences, terminators) and vectors following the standard framework of Golden Gate assembly, which allowed the fabrication of genetic instructions in a combinatorial, low cost and robust manner. In order to image multiple wavelength with a single excitation, we used a series of long stokes shift fluorescent proteins that could be combined with cyan/green fluorescent proteins. We developed Python code to operate the hardware to run time-lapse experiments with automated control of illumination and camera. We designed open-source code in Jupyter to analyze data and extract meaningful biological information.

To demonstrate the applicability of this integral system, we performed a diverse range of imaging assays often used in microbial ecology, microbiology and synthetic biology.

All together, these results demonstrates the successful integration of open source hardware, free software, genetic engineering and customizable manufacturing to obtain a powerful, low cost and robust system for STEM education, research and bioengineering. All the resources here developed are available under open source licenses.

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81) Sequencing, de novo assembly and annotation of the transcriptome of the scallop Argopecten purpuratus with focus on immune related genes.

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The scallop *Argopecten purpuratus* is one of the most economically important cultured mollusks on the Chilean coast. Nevertheless, its production has declined in recent years due to disease outbreaks. Thus, the characterization of immune molecular components and a systemic notion of their interaction are required for a functional understanding of scallop immune response. There is a lack of genomic information from this species, therefore, in this work we aimed to characterize de transcriptome of *A. purpuratus* by next generation sequencing and *de novo* transcriptome assembly. For this, 12 cDNA libraries were constructed from gills and digestive gland tissues from naïve and immune challenged scallops and sequenced by the Illumina HiSeq4000 platform. A total of 483,912,924 clean reads were generated, which were *de novo* assembled into 39,499 high quality contigs and subsequently annotated into 11,125 genes (E-value ≤ 1e-25) based on known proteins from the UniProt database, using the CLC Genomics Workbench 10.1.1 software. More than 20 potential immune related gene families were identified using the BLAST algorithm and sequences of interest were confirmed using CDD and SMART databases. Gene ontology (GO) analysis revealed 32 biological process subcategories, 19 cellular component subcategories and 10 molecular function subcategories. By KEGG analysis, 11,125 genes were classified into 23 pathways, among which 3 pathways were related to the immune system. The data presented in this study provides the first insight into the transcriptome profile of *A. purpuratus,* which should be considered for further research in the immune response of the scallop.

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83) Effect of glucocorticoid-mediated stress on the expression of genes involved in teleost skeletal muscle lipid metabolism.

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The stress response in fish is a biphasic process. It starts with an initial release of catecholamines, followed by the secretion of glucocorticoid (cortisol) from the interrenal tissue to the bloodstream. Whereas the stress response axis is highly conserved in vertebrates, cortisol-mediated response in teleosts plays a preponderant role in early metabolic adaptation, however little is known about the effect of stress on the expression of genes involved in teleost lipid metabolism in skeletal muscle. The aim of this study was to evaluate the effect of dexamethasone (DEX), a synthetic GC, on the expression of metabolic genes related to lipid metabolism in rainbow trout. Juvenile rainbow trout (*Oncorhynchus mykiss*) were obtained from Río Blanco pisciculture (PUCV). Fish were used according to protocols preapproved by the Bioethical Committee of UNAB. 24 fish were allocated into 2 groups of 12 individuals each. To stimulate glucocorticoid-induced stress, one group was incubated with DEX, another group with the vehicle (EtOH). After 1, 3, and 5 days of treatment, skeletal muscle was collected and lipid metabolism-related genes (*ppara, atgl, hsl1, hsl2, lpl, acc, fas*) were evaluated by RT-qPCR. Free fatty acid, cholesterol, triglyceride and glucose were also evaluated in plasma. Significant changes were observed in the transcript levels of *hsl1,* three days after the DEX treatment. Furthermore, significant differences were observed in plasmatic levels of free fatty acid and glucose, five days after the DEX treatment. These results suggest that stress is an important regulator of lipid metabolism in teleost skeletal muscle.

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85) ChargaffCracker: a software for cracking the generalized version of Chargaff's 2nd rule.

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During his life, Erwin Chargaff stated two rules for genome sequences: The second rule states that in only one strand of DNA, the number of Adenines (A) is similar to the number of Timines, and the number of Cytosines similar to the number of Guanines.

In the last two decades, it has been proposed that the 2nd rule might be a particular case of a more general rule. The generality has to do with the size k of reverse complement (RC) k-mers for which the rule is valid. For k=1, the RC pairs of 1-mers are A-T and C-G.

Two statistical measures have been proposed to test the generalized Chargaff's 2nd rule (gC2r). Both contain artifacts over the length of the genomes. This has led the authors to think there is a minimum length of a genome and a maximum k-mer for compliance.

In this work, we have developed a statistical test and a measure of compliance of the gC2r that are independent of the size of the k-mer, and the size of the genome.

We have implemented the measures in a software, ChargaffCracker, and with it we hypothesize that:
The compliance of the gC2r is a consequence, not cause of the 2nd rule; While Chargaff's 2nd rule might be a consequence of transpositions and inversions, the gC2r is a property of the sequence model of genomes, not of the biology of organisms. However, this property might have been exapted to fulfill biological needs in genome evolution.

FONDECYT #11140869

87) The role of the mitochondrial ubiquitin E3 ligase 1 (MUL1) in the Mitochondria-Endoplasmic Reticulum (ER) connection of skeletal muscle cells under lipotoxic stress.

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Introduction: Obese, and diabetic patients have increased plasma fatty acids that elicit toxic effects, thus leading to mitochondrial dysfunction and cell death in several tissues, including skeletal muscle. MUL1, originally identified as an E3 protein ligase, has either a small ubiquitin-like modifier (SUMO) ligase activity that stabilizes DRP1 (the main effector in mitochondrial fission), or an ubiquitin ligase activity, degrading MFN2 (a protein regulator of mitochondrial fusion). However, despite the growing interest in the study of the connection between mitochondrial shaping proteins and metabolic regulation, the role of MUL1 in states of lipotoxicity has not been reported.

Methodology: We exposed L6 myoblasts to different palmitate and miristate concentrations to evaluate MUL1 and MFN2 protein and mRNA levels, as well as mitochondrial morphology and mitochondria-ER proximity by confocal microscopy.

Results: In L6 myoblast, miristate and palmitate increased MUL1 protein levels and decreased MFN2, which is consistent with increased mitochondrial fission. Moreover, this mitochondrial fission was accompanied with an increased perinuclear mitochondria-ER colocalization. Finally, the decrease in MFN2 and the increase in the mitochondria-ER proximity were prevented by a MUL1 siRNA.

Conclusion: This data suggests that MUL1 is an important mitochondrial shaping protein involved in the control of the mitochondria-ER connection during lipotoxicity.

FONDECYT 1161156 , PIA 79150007 and FONDAP 15130011.

89) Anti-hypertrophic and metabolic effects of GDF11 on cultured cardiomyocytes.

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Cardiac hypertrophy is a compensatory response to increased work demand and characterized by increases in the heart and cardiomyocyte size associated to increase in contractile protein levels and sarcomere numbers. However, the chronic exposure to neurohumoral stressors such as catecholamines leads pathological phenotype with loss of contractile function and reduction of oxidative energy metabolism (ATP production). Growth Differentiation Factor 11 (GDF11) belongs to TGF-β superfamily and has shown to reverse cardiac hypertrophy in old mice. In vitro, GDF11 also prevented cardiomyocyte hypertrophy induced by phenylephrine. However, these results are controversial. Accordingly, our aim was to investigate in vitro the anti-hypertrophic mechanism of GDF11 in cultured neonatal rat cardiomyocytes. To this end, cardiomyocytes were treated with the pro-hypertrophic agent norepinephrine (NE, 10 uM) in the presence or absence of GDF11 (10 nM) for 48h. Our results showed that GDF11 prevented the increases in cardiomyocyte area and perimeter triggered by NE (evaluated by fluorescence microscopy using rhodamine-phalloidin staining) as well as the increase in protein and mRNA levels of atrial natriuretic peptide (ANP, using Western blot and qPCR, respectively). In addition, GDF11 was able to prevent the decreases on ATP levels and oxygen rate consumption (basal and maximum) induced by NE. Collectively these results suggest that GDF11 prevents cardiomyocyte hypertrophy by controlling mitochondrial energy metabolism.

FONDECYT 1161156 (SL), FONDAP 15130011 (SL), CONICYT PhD fellowship (VG).

91)Changes in synonymous codons usage alter the function of the RNA chaperone Hfq from E. coli.

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The genetic code is redundant, implying that more than one codon can decode the same amino acid (synonymous codons). The choice of synonymous codons present in each gene is not random, as it may affect translation efficiency, as the folding and levels of the encoded proteins, being them selective pressures. In bacteria, translation of mRNAs is regulated in part by sRNA-mRNA interactions in which the RNA chaperone Hfq might participate including processes such as cellular metabolism, virulence, stress adaptation, among others. In order to study the relationship between the use of synonymous codons and the regulation of translation by small RNA in bacteria, we evaluated the effect of synonymous codon replacement on the function of the RNA chaperone Hfq. We replaced synonymous codons in two regions of the *hfq* gene in order to evaluate the effect on translation and folding of the protein. For each modified region generated by random modifications of synonymous codons, *hfq* libraries were produced by homologous recombination. The results suggest that alterations of synonymous codons in the region encoding the carboxyl terminus of Hfq affect cell growth and stress response. We are currently studying the possible effects of synonymous mutations in the translation and folding of the protein.

FONDECYT 1150834

93) B3 transcription factors are involved in iron loading in Arabidopsis embryos.

Grant-Grant S[1], Medina J[2], Vicente-Carbajosa J[3], Curie C[4], Roschzttardtz H[1], [1]Genética Molecular y Microbiología Pontificia Universidad Católica de Chile.[2]Centro de Biotecnología y Genómica de Plantas INIA.[3]Centro de Biotecnología y Genómica de Plantas Universidad Politecnica de Madrid.[4]Developmental Biology CNRS Montpellier. (Sponsored by This Work Was Funded By FONDECYT 1160334 From The Chilean Government, INTER 6809 VRI PUC-Chile And PhD CONICYT-Grant 21170951.)

Introduction: The molecular mechanisms that regulate iron (Fe) allocation in plant seeds are poorly understood. Its know that Fe accumulates in endodermis, a cell layer that surround provasculature, during Arabidopsis embryo maturation. In order to better understand the genetic control of Fe accumulation and distribution in seed embryo, we used mutants in transcription factors known involved in embryo development and seed maturation. We focus on *FUSCA3* (*FUS3*), *ABSCISIC ACID INSENSITIVE3* (*ABI3*)and *LEAFY COTYLEDON2* (*LEC2*) mutants. These genes belong to a B3 containing-domain transcription factors family.

Methodology: We used Perls/DAB stain and Fe quantification by ICP-MS to evaluate Fe distribution and content in mutant and wt *Arabidopsis thaliana* embryos. qRT-PCR analysis was performed in order to determine changes in expression of key genes. **Results:** We observed that these mutants have different distribution and content of Fe in embryos. When we analyzed Fe distribution in *fus3,* we could not observe Perls/DAB stain, for *lec2* we only could observe stain in radicle but not in cotyledons, and for *abi3* we observe the same stain pattern found in wt embryos, but with less intensity. We measure the content of Fe in seed, *abi3* shows less content of Fe that wt seeds. Finally, we observe that several key genes in Fe homeostasis have altered expression in *abi3.*

Conclusion: B3 containing-domain transcription factors family have an important role in accumulation and distribution of Fe in *Arabidopsis* embryos. Finally, it seems that this transcriptions factors could be involved in the regulation of Fe uptake in embryos.

95) Evaluation of the efficacy of antisense therapy in a patient derived xenograft model (PDX) of advanced cervical cancer.

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Carcinoma of the cervix remains a significant health problem for women worldwide and new treatments are urgently required. In previous works we have described that the interference of Antisense non-coding mitochondrial RNA (AsncmtRNA) with antisense oligonucleotides (ASO) induces a massive cell death mediated by apoptosis in primary cultures of human advanced cervical cancer.

The aim of this study was to evaluate the efficacy of the ASO treatment using patient derived xenograft (PDX) models of advanced cervical cancer. Therefore, fresh human biopsies were surgically implanted into the cervix of immuno-compromised mice (F0). One hundred days after the engrafted, tumor was harvested and fragmented to expand subcutaneously into a new group of mice, referred as F1. Animals were treated with 200 µg of AS0 1537S, every other day until complete 10 doses.

Primary biopsies and tumor growth in the different generations, were HPV-genotypes by PCR and, a broad analysis of proliferation, antiapoptotic, epithelial and angiogenesis biomarkers were carried out by western blot and immunohistochemistry.

The characterization of tumor samples determined the presence of HPV 16 and no changes in expression of Cytokeratin 17, PCNA and Ki67 were observed through the different generations. The evaluation of the ASO therapy do not show a delay on tumor growth but shows an important inhibition of the metastatic spread, associated with a drop in the expression of Survivin, Rac and Bcl-xL.

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97) Deriving evolutionary relationships between the membrane coat proteins based on structural comparisons.

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Membrane coat proteins (MC) are indispensable for intracellular traffic and they predominate in the eukaryotic endomembrane system. Phylogenetic and comparative genomic analyses have suggested that MC proteins arose by duplication and divergence from the trafficking machinery that was present in the Last Eukaryotic Common Ancestor (LECA). The “protocoatomer” hypothesis proposes that these proteins share a common ancestor based on structural information. However, the MC proteins display a wide variety of forms and a large sequence divergence among them, which makes difficult the reconstruction of the evolutionary intermediate steps of the MC proteins with the current comparison tools based on structural and sequence alignments. We previously developed an efficient computer program, called MOMA (for MOrphing & MAtching) to achieve a flexible and global comparison of protein structures based on matrices of the secondary structure elements (Gutiérrez et al. 2016). We have implemented a new methodology to evaluate the significance of local structural alignments found with MOMA based on Perceptron analysis. In addition, we improved their superpositions using the iterative closest point algorithm (ICP) and report the best combination of the local alignments with the STOVCA software. Application of our method allowed us to confirm the known relationships previously described in the literature and also revealed unexpected new relations among the MC proteins. Our results support the evolution of the protocoatomers by duplication and divergence, thus reinforcing the proposal that the MC proteins have played an important role in the emergence of the complexity in eukaryotic cells.

This work was funded by CONICYT PhD fellowship, FONDECYT 1141172 and CONICYT PIA ACT1408.

99) Inhibition of DRP1-dependent mitochondrial fission prevents hypoxia-induced metabolic shift and cellular proliferation of pulmonary arterial smooth muscle cells (PASMC).

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Introduction: Pulmonary arterial hypertension (PAH) is a chronic progressive disease characterized by excessive proliferation of the PASMC, thereby reducing the lumen of pulmonary arteries and leading to poor blood oxygenation. Recent studies revealed a role of mitochondria in PAH pathogenesis, as key regulator of cell bioenergetics and survival. In this work, we assessed whether hypoxia-induced mitochondrial fission and specifically the fission protein DRP1 are responsible for the hyperproliferative PASMC phenotype.

Methodology: We evaluate whether the pharmacological inhibitor of DRP1, Mdivi-1, or a DRP1 dominant negative (*K38A*) can prevent PASMC metabolic shift and proliferation in an *in vitro* hypoxic model of PAH.

Results: We showed that prolonged hypoxia (48 h) induces mitochondrial fragmentation along with an increase in the levels of DRP1. Concomitantly, we observed a drop in oxygen consumption and higher extracellular lactate levels, together with increased cell proliferation. Aside from restoring mitochondrial morphology, Mdivi-1 prevented all these changes, whereas the DRP1 *K38A* avoided the hypoxia-induced proliferation as well.

Conclusion: Our data suggest that DRP1-dependent mitochondrial fission is a new pharmacological target suitable for the treatment of PAH.

FONDECYT 1141198, PIA 79150007 and FONDAP 15130011.

101) A three-dimensional structural model of resveratrol O-methyltransferase (VvROMT) of Vitis vinifera.

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Stilbenes are phenolic secondary metabolites, which play an important role in the defensive response of plants. Resveratrol is one of the most studied stilbenoids, due to its beneficial effects for human health, but suffers from low bioavailability. Therefore, more stable derivatives with improved bioactivity are desirable for large-scale industrial production. It has been shown that a modification of the hydroxyl moiety of stilbenes by O-methylation or glycosylation increases solubility, stability and uptake into human cells. The enzyme resveratrol O-methyltransferase (VvROMT) catalyzes the dimethylation in *meta* position to obtain pterostilbene. Nevertheless, few plant enzymes have been identified to be able to methylate resveratrol in *para* position. Due to its favorable catalytic properties, we propose VvROMT as a candidate to change regiospecificity through rational protein engineering. However, the enzyme's three-dimensional structure has not been resolved yet. Here we present a three-dimensional model of the homodimeric structure of VvROMT in complex with its co-factor S-adenosylmethionine and substrate compounds. The model was created with MODELLER, followed by loop refinement with ROSETTA and assessed through the SAVES server. Docking was performed with different substrates using GOLD. We observed that the highest docking score was concordant with the native substrate of the enzyme and that it was accommodated in a catalytically competent conformation. Molecular dynamics simulation is ongoing to validate their interactions and structure flexibility. The model will be useful to identify key residues that allow us to change its pattern of methylation and obtain the improved antioxidant trimethoxy-resveratrol. ACKNOWLEDGEMENTS: Proyecto-INTERDISCIPLINA-VRI-UC-II160020

Beca CONICYT Doctorado 2016.

103) What is killing the bees? A transcriptomic and proteomic approach.

Himelreichs J[1,3], Haro R[3], Molina C[2,3], Manzi C[3], Vergara J[3], Tatham M[4], Hay R[4], Rojas-Fernandez A[5,1,4], Silva A[3,2], [1]Center for Interdisciplinary Studies on the Nervous System (CISNe) Universidad Austral de Chile.[2]Institute of Biochemistry and Microbiology Universidad Austral de Chile.[3]AUSTRAL-omics Universidad Austral de Chile.[4]Center for Gene Regulation and Expression , College of Life Sciences, University of Dundee.[5]Institute of Medicine Universidad Austral de Chile. (Sponsored by Proyecto Fondecyt de Iniciación (#1114068-AXS); Beca Conicyt de Magister Nacional Año 2015 (#22150155-JH, #22151110-RH) y Año 2017 (#22171413-CM)).

During the last decade, the decrease in pollinators in both quantity and diversity has caused worldwide concern, which can be seen in the case of the honeybee (*Apis mellifera*) as the sudden loss of worker bees in managed hives without an obvious cause. This decline, also known as the Colony Collapse Syndrome (CCD), appears to be triggered by several factors, within which the use of neonicotinoids, a class of neuro-active pesticide, has played a key role. After being applied in seeds or aerial form in the field, these substances remain long time in the environment and are transported and bio-accumulated by the bees in the hives. There is scientific evidence that the physiological effect of these substances is negative, affecting the nervous system of bees in many ways, but the physiological effect of small doses is still uncertain. In this work we studied the effect of residual doses of imidaclorid (2,5 ppb), a worldwide used neonicotinoid, on *A. mellifera*. We analyzed transcriptomic and proteomic responses through a comparison of adult synchronized individuals from exposed and unexposed hives to the toxic compound. We obtained the mRNA from heads, and we applied massive sequencing together with a proteomic strategy of total protein lysis and peptide analysis by LC-MS / MS, followed by bioinformatic and statistics analysis for each of the areas covered.

Keywords: *Apis mellifera*, Residual dose, Neonicotinoids, Transcriptomic, RNA-seq, Proteomics, LC/MS-MS.

Proyecto Fondecyt de Iniciación (#1114068-AXS)

Beca Conicyt de Magister Nacional año 2015 (#22150155-JH, #22151110-RH)

Beca Conicyt de Magister Nacional año 2017 (#22171413-CM)

105) Iron distribution during seed development in arabidopsis thaliana and brassica napus.

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Iron is an essential micronutrient for most living organisms. The role of iron in seed yield is an important agronomical trait because iron deficiency affects plant reproduction and crops yield. Also, according to the World Health Organization, 30% of the population is anemic, and biofortification is a possible alternative to combat iron deficiency, and seeds with higher mineral content may contribute to this goal. One of our main goals is to understand the molecular basis of iron homeostasis during seed development in plants. Using *Arabidopsis thaliana*, it has been described that iron accumulates in vacuoles of endodermis cell layer during embryo maturation. Using Perls/DAB staining we were able to find that embryos from related species to Arabidopsis have a different iron distribution pattern. In those embryos, iron accumulates in several cell layers in hypocotyl, including endodermis and at least one layer of cortex. Interestingly, cotyledon of those embryos accumulates iron only in the endodermis cell layer. In addition, we evaluate iron accumulation during seed development and we found that iron accumulates in endosperm nuclei prior to reach the embryo in early stages of development of *Brassica napus* seed. Our results open new questions about the molecular mechanism controlling iron loading in seeds.

107) Structural characterization of FcEG1; an endoglucanase enzyme involved in fruit softening of Fragaria chiloensis.

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Endo-β-1,4-glucanases (EG) have roles in synthesis, remodeling and degradation of the cell wall. EG enzymes are associated to several processes in plants including fruit ripening. Changes in the cellulose-hemicellulosic fraction have been previously reported during ripening, and match with softening of *Fragaria chiloensis* fruit. A full-length sequence of *FcEG1* was obtained; the transcript accumulation of *FcEG1* was determined using qRT-PCR. Phylogenetic analyses suggest that *FcEG1* belongs to α group of GH9 family with other proteins previously described with roles in elongation, ripening, and abscission.To gain insight about the protein structure of FcEG1 and its mechanism of action at the molecular level, the protein model was built, and was validated and refined through molecular dynamics simulation (MDS). The model obtained display a β-barrel–type structure that is typical of GH9 enzyme family that comprises 12 α-helix, 2 3_{10} helices and 6 β-sheets; and an open groove in the center of the enzyme where the catalytic residues is oriented to the solvent. The interaction of FcEG1 with a set of putative substrates was explored using MDS. The results of this interaction suggest the formation of the a stable conformational complex between FcEG1 and the XXXG substrate a type of xyloglucan. Additionally, an *in silico* site-directed mutagenesis studies were carried out, our study indicates that Asp253 and Tyr448 residues are important in the binding of the ligand. Thus it can be concluded that FcEG1 relevant in the cell wall remodeling during fruit ripening.

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109) Generation of Nanobodies: A local weapon against local diseases.

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The Chilean natural barriers and the mountainous landscape allowed the development of many endemic animals, including several species of camelids such as Alpacas, Llamas, Vicuñas and Guanacos. In this work, we aim to use the extraordinary nature of the Camelid immune system to generate immunotherapies and diagnostic tools against emergent virus infections such as the Hantavirus. In contrast to other mammals a large proportion of Camelid antibodies (up to 80%) belongs to single domain antibodies (or Heavy chain only antibodies, HCAbs), an antibody type lacking the light chains and displaying its antigenic affinity from a single polypeptidic sequence. Interestingly, HCAbs are exclusively found in Camelids and some Sharks species. HCAbs are made of two identical heavy chains, each divided in two regions: a constant domain knows as fragment crystallisable region (Fc region) and a variable region responsible for its binding to cognate antigens known as VHH and commercially called Nanobodies. Nowadays, it is possible to immunize camelids using recombinant proteins to direct the immunologic response against specific antigens such as disease-related targets and/or commercially interesting proteins. Upon immunization with a target protein the blood of camelids gets enriched with HCAbs and HCAbs-producing B-lymphocytes. After immunization it is possible to extract the B-lymphocytes to further isolate the cDNA region coding the VHH (i.e. the Nanobodies). Nanobodies can be used TO NEUTRAlise virus infections, poison from snakes and spiders as well as for diagnostic.

111) Structure-based discovery of novel adamantane-based selective 11beta-HSD1 reductase inhibitors.

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Overexpression of 11**β**-HSD1 in key metabolic tissues is related to the development of type 2 diabetes, obesity, hypertension and metabolic syndrome. Using crystal structures of human 11**β**-HSD1 in complex with inhibitors as source of structural information, a structure-based virtual screening approach was implemented to identify novel 11**β**-HSD1 inhibitors. A selected group of compounds was identified in silico and further evaluated in cell-based assays for cytotoxicity and 11**β**-HSD1 mediated cortisol production inhibitory capacity. Biological evaluation in adipocytes and steroid quantification by HPLC-MS/MS identify 2 compounds that exhibit 11**β**-HSD1 mediated cortisol production inhibitory activity with potencies in the micromolar range and selective against 11**β**-HSD2. In vitro and in silico characterization of ADME/Tox properties were performed to predict the likelihood of the candidate compound to survive successive stages of development. Activity of selected compounds on cytochrome P450 enzymes was investigated to establish their potential for interactions with other drugs, demonstrating that the compounds are weak inhibitors of the CYP450 system and do not induce their activity. Determination of plasma protein binding was performed for human serum albumin (HSA), **α**1-acid glycoprotein (AGP), the compounds display medium to high plasma protein binding percentages (> 90%). Two novel adamantane-based compounds showed to be selective for the reductase activity and over 11**β**-HSD2 isoform, and thus represent novel leads for the development of more active derivatives with improved solubility and pharmacokinetic profile **targeting intracellular cortisol levels in type** 2 diabetes and metabolic syndrome.

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113) Hsp70 Immunoglobulin binding Protein (BiP) viscoelastic properties determined by nanorheology and its functional characterization.

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BiP is a chaperone belonging to the Hsp70 family, that is involved in the regulation of important biological processes such as synthesis, folding and translocation of proteins in the endoplasmic reticulum. This chaperone has two domains communicated between each other that modulate the catalytic activity; a Nucleotide Binding Domain (NBD), in the N-terminal extreme, which binds and hydrolize nucleotides, and the Sustrate Binding Domain (SBD) in the C-terminal extreme, which binds the extended conformation of polypeptides. We will study the mechanical properties of BiP associated with the binding of their substrates directly by nanorheology. In this method BiP protein is anchored between a layer of gold nanoparticles (GNPs) and a microscope slide coated with a thin gold layer through specifically modified cysteines in NBD V166C and SBD G518C. This technique shows if changes in the dissociation constant (KD) of the protein are related to its elastic properties, and the relation that exists between the binding of the substrate and the protein's mechanical properties. Preliminary results show that the protein gets softer when binds nucleotide, whereas with peptide substrate it gets stiffer. On the other hand, we established a method to measure catalytic activity of BiP using a coupled assay to NADH oxidation. Once BiP catalyze ATP hydrolysis, the product formed, ADP, triggers a series of reactions that lead to NADH oxidation which can be quantified spectrophotometrically at 340 nm. We will determine the Michaelis constant (KM) of ADP and correlate this value with the KD obtained with nanorheology.

FONDECYT 11130263, PCI PII20150073

115) Participation of Polycomb group proteins and JMJD3 demethylase on the transcriptional regulation of the INK4A/ARF locus and CDKN1A gene in hippocampal culture neurons

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Cellular senescence is a process that is activated after a finite number of cell divisions, in order to prevent an excessive proliferation of cells. It has been reported that Polycomb Group proteins (PcGs) are able to negative regulate the expression of proteins associated with the senescence process, at the *Ink4a/Arf* locus. Therefore, a decrease in the expression of PcGs genes results in a significant increase in the levels of $p16^{Ink4a}$ and $p14^{Arf}$ proteins, triggering a premature senescence process in replicative cells. However, are still remain dilucidated if this process occurs in post-mitotic cells as neurons. We postulated that PcGs proteins have a repressive regulatory effect on the transcriptional control of the *Ink4a/Arf* locus and the *Cdkn1a* gene, during senescence in hippocampal neurons. We performed shRNA assays against Ezh1 or Ezh2 PcG methylases or JMJD3 H3K27me3 demethylase in hippocampal cultures at 5 and 15 days *in vitro* (DIV), generating a decrease in its expression. After shRNA treatment, we analyzed *Ink4a/Arf* and *Cdkn1a* mRNA levels observed that a decrease of Ezh1 or JMJD3 expression correlates with $p16^{Ink4a}$ and $p21^{Cip}$ expression decrease in hippocampal cultured neurons at 15DIV, suggesting that Ezh1 and JMJD3 plays an important role on the transcriptional regulation of the *Ink4a/Arf* locus and the *Cdkn1a* gene in hippocampal cultures.

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117) Synergistic effects of Paraburkholderia phytofirmans and Azospirillum brasilense on growth and stress tolerance of Arabidopsis thaliana plants

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Plant growth promoting rhizobacteria (PGPR) have been shown to improve plant growth and tolerance to environmental stress under different experimental systems, involving the interaction of a single bacterial strain with a plant host. However, little is understood about the simultaneous interaction of the plant with more than one beneficial bacterium, as occurs in a natural rhizosphere environment, and the possibility that this results in complementary, or synergistic microbial effects on plant growth. *Paraburkholderia phytofirmans* PsJN and *Azospirillum brasilense* Sp7 are two well-studied PGPR, that stimulate growth and tolerance to saline stress in *Arabidopsis thaliana*. We have shown, however, that bacterial phytostimulation mechanisms differ among the two species, and their effects on Arabidopsis development can be clearly differentiated. Here, we tested co-inoculation of both bacteria in gnotobiotic plant growth chamber *in vitro*, and measured microbial growth through time, colonization patterns, plant development, stress tolerance and expression of IAA, ABA and JA pathway gene markers, in order to assess the potential onset of competition, complementation or synergism among the bacterial strains. The results of this work show that bacterial competition exists at the rhizosphere level, where *P. phytofirmans* is by far the predominant species, but both bacteria are able to exert their effects on the host, producing complementary and even synergistic beneficial responses on the plant. We conclude that combined inoculation of bacteria that activate plant growth through different pathways can produce a stronger developmental impact and higher stress tolerance in plants than inoculation of individual PGPR.

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119) Physiological consequences of changes in tRNA^{Gly} activity during oxidative stress

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Bacteria continually use mechanisms to tolerate oxidative stress produced by diverse environmental factors. The study of this response has focused in transcriptional changes controlled by OxyR and SoxR together with other transcriptional factors, but there is very little knowledge about a potential role of the translation machinery. A screening developed in our laboratory showed a specific decrease in active tRNA^{Gly} when *Escherichia coli* is exposed to oxidative stress. This condition does not alter the levels of RNA, but decrease the aminoacylated fraction of all tRNA^{Gly} isoacceptors *in vivo* suggesting their inactivation. Using a fluorescent reporter, we have found that glycine codons change their efficiency of translation under oxidative stress, and that this effect depends on the availability of glycine in the media. Additionally, over-production of some tRNA^{Gly} isotypes alters the production of diadenosine tetraphosphate (Ap4A) under oxidative stress, a metabolite produced by aminoacil-tRNA synthetases under some stress conditions. Interestingtly, over-production of tRNA^{Gly} also alters diverse phenotypes potentially related to the accumulation of Ap4A, like bacterial growth, motility and fermentation of carbohydrates. This suggests that changes in tRNA^{Gly} produced by oxidative stress may be transduced through Ap4A and changes in the rate of translation in some codons. Based on all this data, we propose that changes in levels of active tRNA^{Gly} contribute to the response to oxidative stress by regulating changes to the bacterial proteome and physiology.

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121) Study of the effect of vitamin k analogues on cell viability

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(Sponsored by Fondecyt 1150822 - 1150822)

Vitamin $K_3$2-methylnaphthalene-1,4-dione (Menadione) is an important metabolite of vitamin K_1. Menadione is a molecule which acts as a co-factor in blood coagulation cascade, and in bone mineralization, as well as an electronic mediator in the respiratory chain. Besides, Dichlone is a derivative has been used as agrochemical, with insecticide and antifungal activities. Studies suggest that the addition of substitutes in position 3 of the naphthoquinone ringconfers differential biological activity to these compounds, including the potential to interact with the active site of acetylcholinesterase, a molecular target for the development of several insecticides. Here we describe the synthesis of different compounds derived from Dichlone with potential cytotoxicity activity on insect cells, which could be explained by an interaction with the enzyme acetylcholinesterase (AChE). We assessed toxicity of these compounds on Schneider 2 (S2) cells. This is a cell line derived from embryos of the fly *Drosophila melanogaster*. This is an insect model used to identify molecular targets of new chemicals with potential as insecticides, manipulate insecticide resistance genes, and also to investigate the interactions between ligand and the proteins they target. To determine the possible interaction between the compounds and *Drosophila* AChE (DmAChE) *docking* studies were performed (DmAChE PDB code 1DX4). The docked compounds were stabilized in the cavity by forming different types of interactions including hydrogens bonds, π-π stacking interactions. In addition, the cytotoxic effect (IC_{50}) on S2 cells was determined in *in vitro* assays. Our data suggest that some moieties enhance cytotoxic properties of these compounds.

123) Quantitative proteomic analysis of SUMO modification rearrangements upon starvation: An new role for SUMO.

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

125) New system of PCR primer design allows identifying circulating microRNA-15b as a Potencial Bio-Marker in Non-Ischemic Heart Failure.

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The use of data mining strategies on public access databases is an innovative approach to identify potential biomarkers in complex diseases such as heart failure (HF). We assessed the hypothesis that a bioinformatic strategy of meta-analysis using existing public databases might predict novel differentially expressed circulating microRNA in non-ischemic HF patients.

We systematically reviewed the literature for microRNA profiling in HF (2006-2014). Raw data from the largest study was normalized and analyzed by unsupervised hierarchical clustering. Significance analysis selected microRNAs with a fold change of 2 and a false discovery rate of less that 1%. Significant microRNAs in the other studies were incorporated in a meta-analysis using a robust rank aggregator approach. To validate the in-silico analysis, we evaluated the microRNAs with higher predicted fold change in plasma samples from 23 patients with HF and five healthy controls using RT-qPCR through of design of primer that increase the size of amplicon of microRNAs.

We identified 54 microRNAs that fulfilled our criteria. The meta-analysis confirmed nine differentially expressed microRNAs, including the previously reported let-7b, miR-199a and miR-23a and 3 newly associated microRNAs (miR-125b, miR-140, and miR-15b). We validate our designed primers by RT-qPCR. HF plasma samples revealed that both miR-15b-5p(p=0,004) and miR-199-3p(p=0,008) were reduced in HF subjects. Conversely, miR-23a(p=0,0021) and let-7b(p=0,0195) were increased in HF subjects

Designed primers allowed exploring multiple microRNAs. Bioinformatic analysis allows the identification of previously unreported microRNA associated with HF. This novel approach, using publicly available data, may accelerate the pre-analytic phase of biomarker research.

FONDAP ACCDiS 15130011 (Castro P. and Corvalán A.), FONDECYT 1141198 (Castro P.), FONDECYT 1150359 (Verdejo H.) and FONDECYT 11140470 (Quiroga C.).

127) Distinguishing coding and non-coding sequences and RNA families assignation through machine learning approaches.

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Non-coding RNAs are important players of cellular regulation. Two key steps on the predicting process and functional assignation of ncRNAs are the ability to distinguish coding/non-coding sequences, followed by a functional assignation of RNA families. Here, we applied different machine-learning approaches (Naive Bayes, SMO, IBK, Random Forest) to predict the coding potential and functional assignation of RNA families, based on nucleotides analyses. To distinguish coding/ncRNAs, we randomly selected sets of ncRNA sequences, extracted from Rfam; and coding genes from human RefSeq. Firstly, all ncRNAs were filtered using a maximum-similarity cutoff of 80% (Levenshtein distance). Then, coding/ncRNAs had their mono-, di- and tri-nucleotides counts were analysed through all algorithms using three equally divided sets of 200, 400 and 1000 sequences. Coding/ncRNA sequences from six model organisms were used to measure methods performance. The RNA family assignation was performed using multiple-alignments of: 100 sequences from 10 different families; 200 sequences from 20 families; three different sets of 500, 1000 and 2000 sequences from 50 families. The coding potential evaluation using 200, 400 and 1000 sequences presented accuracies ranging 99-99.2%. Analyses with model organisms reached accuracies of 95%. Functional assignation of RNA families using 10 and 20 families, revealed results with accuracies reaching 99% and 98.5%, respectively. Using 50 families, the best accuracy was 94.2%. Family assignation using specific sequences of taxonomic-related organisms reached accuracies ranging 99-100%. Our results outperformed those from literature, opening opportunities for the development of improved tools for ncRNA analyses.

FONDECYT Iniciación 11161020

129) Rlf protein as a epigenetic regulator in the adaptative mechanism of Orestias ascotanensis.

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Orestias ascotanensis is a fish that inhabits in high altitude areas of the Chilean Altiplano, where species have adapted to high salinity, UV radiation and temperature fluctuation, among other extreme conditions. Thus, *Orestias* is an interesting specie to study the mechanisms of adaptation to extreme environments.Studies in other fishes have shown that long-term adaptive changes occur at a molecular level. In *Orestias*, however, it remains to be determined what are the molecular mechanisms that support its ability to rapidly adapt to extreme environments; We propose that this process is associated with regulation at the epigenetic level. In this work, we have identified the transcriptional regulator RLF as a potentially relevant component in the epigenetic mechanisms that control gene expression associated with adaptation of Orestias to its changing environment. RLF was selected based on three specific bioinformatics criteria following transcriptomic analyses of total RNA samples (microRNAs and mRNAs). First, we identified 393 genes showing positive selection during our genome analysis. Interestingly, among them we found enrichment for metal and protein binding functions. Second, we selected genes coding proteins with zinc fingers domains as they represent factors tightly associated with transcriptional responses to stress conditions. Third, we identified genes that are miRNA targets as this regulatory mechanism exhibits unique features in fishes. RLF was the gene meeting these three criteria. The presence of RLF in fish samples was further confirmed by qPCR and Western Blot. These results, will now allow the identification of RLF target regulatory regions in *Orestias* genome.

131) Activation of the AT2 receptor blocks Caveolin-1 enhanced melanoma migration and metastasis.

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Introduction: Metastasis is responsible for most cancer-related deaths. Recently our group discovered a new pathway involving the proteins Caveolin-1 (CAV1), Rab5 and Rac-1 (CAV1 / Rab5 / Rac1 pathway) that, upon activation, promotes migration, invasion and metastasis. The Renin-Angiotension system is responsible for regulating systemic blood pressure, but more recently has also been implicated in controlling metastasis of cancer cells via receptors of this system. Specifically, the activity of one these receptors, AT2 (ATR2), has been linked to suppression of metastasis by inhibiting signaling pathways involved in cell migration. Here, we evaluated the possibility that the anti-metastatic effect of AT2R may be due to inhibition of the CAV1/Rab5/Rac1 pathway in melanoma (B16F10) cells.

Materials And Methods: Expression of AT2R and activation of downstream targets in B16F10 cells was measured by Western blotting. Cell migration was evaluated in Transwell assays. Rac1 y Rab5 activation were assessed using PBD pull-down assays. Lung metastasis of B16-F10 cells was evaluated in syngenic C57BL/6 mice.

Results: B16F10 cells express a functional AT2R. Activation of this receptor decreased phosphorylation of CAV1 on tyrosine-14 and Rab5/Rac1 activation. Moreover both CAV1 enhanced B16F10 migration and metastasis were diminished.

Conclusion: Activation of AT2R reduces the metastasis of B16F10 melanoma cells by inhibition of the CAV1 / Rab5 / Rac-1 pathway.

133) Phycobiliprotein extracted from Gracilaria chilensis as photosensitizers in DSSC.

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Gracilaria chilensis have protein complexes called phycobilisomes which are composed of Phycoerythrin (PE), Phycocyanin (PC), and Allophycocyanin (APC). These Phycobiliproteins (PBPs) possess high absorption coefficients in the visible light (range 500-650 nm) and high quantum yield in fluorescence, making them excellent candidates for the sensitization of semiconductors for dye sensitized solar cell (DSSC) application. It is therefore of great interest to study the usefulness of *G. chilensis* phycobiliproteins as photosensitizers.

Phycoerythrin, Phycocyanin and Allophycocyanin were purified by anion exchange chromatographic method. Photoelectrodes were prepared using these proteins as sensitizers by tinting titanium dioxide (TiO_2) nanoparticles (NPs) grown over fluorine-doped tin oxide (FTO) glass. Sensitization was done twice with 0.5 mg/mL of protein for 24 hours. The cell was assembled using Iodide/triiodide as the electrolyte and platinum as the counter electrode. Efficiency of the assembled device was studied using a Solar simulator (under AM1.5 illumination, 100 mW/cm^2) incorporated with a Keithley source meter.

Even though the efficiencies were found to be low; 0.023 % for PE, 0.025 % for APC, and 0.027% for PC, PBPs increase the efficiencies of the Titanium dioxide in 38%, 58% and 68% respectively. Thus, these Phycobiliproteins have proven to function as sensitizers for photon to electron conversion. The low efficiency of the PBP-DSSC could be due to low adsorption of PBPs onto the TiO_2. Studies are ongoing to increase sensitization using cross linking agent.

VRDI-Enlace : Nº 216.037.021-1.0.

S. Ramkumar (Fondecyt Postdoctoral program N° 3150213).

M. Paulraj (Fondecyt Regular N° 1130802).

135) Study of the subcelular localization of herpud1 during osteoblastogenesis.

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Introduction: Bone is a dynamic tissue whose integrity depends of a balance among synthesis by osteoblasts and resorption by osteoclasts. Osteoblastogenesis involve a major demand of biogenesis, folding, assembly, trafficking, protein degradation and therefore a strict control of proteostasis. The homocysteine-inducible ER protein with ubiquitin-like domain 1 (HERPUD1) is an integral endoplasmic reticulum (ER) membrane protein that is up-regulated under ER-stress by the unfolded protein response (UPR). HERPUD1 play a crucial role in ER-associated degradation (ERAD). In our laboratory, we observed that during osteoblastogenesis HERPUD1 expression was increased. The aim of this work, is to study the localization of HERPUD1 and its co-localization with ER-markers during osteoblastogenesis.

Methodology: Pre-osteoblastic cells MC3T3-E1, were growth in MEM-alpha 10%FBS and differentiated with addition of Ascorbic acid, Dexamethasone and beta-glycerol phosphate during 3 and 7 days. HERPUD1 levels was detected by Western blot. For immnunofluorescence, cells were fixed and incubated with antibody against HERPUD1 and ER markers. Fluorescence microscopy was carried out using a Confocal microscope (Zeiss LSM 510), and the images was analyzed through 3D-reconstruction and co-localization analysis using ImageJ software.

Results: Our results shown that according to the literature, osteoblasts increase the size during osteoblastogenesis and immunofluorescense analysis shown that HERPUD1 has an ER localization, determined by co-localization with PDI (ER-marker) in the perinuclear region, and its distribution is spread to the cell periphery during osteoblastogenesis.

Conclusion: These results suggest that, the overexpression and spread distribution of HERPUD1 during osteoblastogenesis could help to extend the control of proteostasis in this cell.

FONDECYT 11140470 (CQ)

137) Comparative study of the metabolic activity of the gibberellin oxidases in rhizobacterias isolated from phaseolus vulgaris nodules.

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The indiscriminate use of synthetic fertilizers in agriculture has aroused the growing interest in finding a replacement of these fertilizers to improve the productivity of agricultural crops without degrading the environment. The plant growth promoting rhizobacteria (PGPR), are outlined as the great candidates for their potential use as biofertilizers, since these microorganisms besides fixing N_2 and phosphates in a symbiotic state also synthesize some plant growth promoting phytohormones such as gibberellins (GAs), a diterpenoid present in higher plants, some fungal and bacteria. In contrast to plant and fungal, few information is available about the enzymes and reactions of GA biosynthesis in bacteria. GA oxidase activities have been detected at significant levels only in bacteroids of *Bradyrhizobium diazoefficiens*, a rhizobacteria that contains an operon of GA biosynthesis genes that is expressed under the microaerobioc conditions found in root nodules. In this work the oxidase activies were investigated in two *Rhizobium* symbionts of common bean: *Rhizobium leguminosarum* sv.*phaseoli* and *Rhizobium etli*. Bacteroids were obtained from symbiotic root nodules of plants inoculated with each of these species and ^{14}C-Labelled GA precursors were added to a bacteroid suspension. The metabolization products were isolated and identified by HPLC. In the different strains, the oxidase activities showed a different behavior in the time range studied. Therefore, both rhizobacteria possess the capacity to metabolize GAs precursors, presenting *R. etli* a greater metabolic activity.

139) Viral Genome-Linked Protein (VPg) Is Essential for Translation Initiation of Infectious Pancreatic Necrosis Virus (IPNV).

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Infectious pancreatic necrosis (IPN) is a contagious viral disease affecting salmonids, causing large economic 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 during its translation. However, there are not studies about the regulation of viral mRNA translation. We demonstrate that the mRNAs are covalently linked to VPg and that Vp1 binds eiF4E. to analyze the implication of VPg in the translation of the viral mRNA, we performed treatment with proteinase K on the mRNA-A and mRNA-B and analyzed the effects on the translation in Rabbit Reticulocytes Lysate system. Additionally, we performed studies of competition of capped mRNA against recombinant Vp1. These results strongly suggest that VP1 acts as a proteinaceous substitute of the cap.

Proyecto Fondecyt N° 1150901; Doctorado De Microbiología, Universidad De Santiago De Chile. Becas De Arancel Postgrado, Vicerrectoría Académica.

POSTER 2

2) In silico analysis of transcriptional regulation in arabidopsis thaliana over-expressing a pinus radiata d. Don mads10.

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The response to inclination in trees is a widely studied phenomenon, but the molecular mechanism involved are still unclear. A MADS-box transcription factor was found differentially expressed in response to inclination (2 hours after treatment) in *Pinus radiata* D. Don. We overexpressed this MADS10 gene in *Arabidopsis thaliana* and identified up and down regulated genes using the Affymetrix AraGene chip. A total of 1211 genes were modulated, with 689 genes up regulated and 529 genes down regulated. Biologicals processes were analyzed using the BioMaps from VirtualPlant. An overrepresentation of transcripts belonging to response to stimulus was observed, within this group response to starvation, cell communication, regulation of biological process were mainly identified. MapMan was used to complement and create a general view of differentially expressed genes. We analyzed in silico the elements in putative Cis binding to MADS-box with PlanPan 2.0 and Jaspar. We build a network with GeneMania to observe the co-expression and interaction between them. Finally, we observed a hierarchical relationship from *PrMADS*10 to other transcription factor families like bZIP, HB and AS2 for up regulated; and, C2C2-CO-like, WRKY, JUMONJI and MYB for down regulated.

4) Angiotensin-(1-9) attenuates ischemia/reperfusion damage in isolated adult rat heart.

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Introduction: Insufficient supply of blood to the heart (ischemia, I) leads to myocardial death. Paradoxically, reperfusion (R) of ischemic myocardium exacerbated cardiac death. In the recent years, angiotensin-(1-9) [Ang-(1-9)] via angiotensin type 2 receptor (AT2R) and angiotensin-(1-7) [Ang-(1-7)] via Mas Receptor (MasR) have shown to antagonize the overactivation of the renin-angiotensin system. Although Ang(19) has anti-hypertrophic action, it remains unknown if this peptide can exert cardioprotective effects.The objective of this study wasto assess whether Ang-(1-9) exerts protective effects in the rat heart subjected to I/R.

Methodology: Isolated rat heats were subjected to global I/R for 30 and 60 min, respectively. Ang-(1-9) was administered with or without A779 (MasR antagonist) or PD123319 (AT2R antagonist). The left ventricle developed pressure (LVDP) and the maximal positive peak of first derivative of LV pressure (+dP/dtmax) were also assayed. At the end of experiment, hearts were perfused with TTC to measure infarct size.

Results: Treatment with Ang-(1-9) during reperfusion improved the recovery of LVDP and +dP/dtmax compared with hearts without treatment (from 10% to 60%, n=5, $p<0.05$). Ang-(1-9) also attenuated myocardial infarct size compared with hearts without treatment (from 42% to 12%, n=5, $p<0.05$). These cardioprotective effects of Ang-(1-9) were blocked by the AT2R antagonist (PD123319), but not by A779**.**

Conclusion: Ang-(1-9) attenuates the damage triggered by I/R in isolated rat heart by an AT2R-dependent mechanism.

EM hold PhD CONICYT and COLCIENCIAS fellowship. FONDEF D11I1122 (MPO; SL), FONDAP1 5130011(MPO; SL).

6) A sequence order-independent clique-matching approach for the comparison of protein binding sites.

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Predicting the macromolecular targets of small molecule compounds is important for drug discovery in order to flag off-targets, identify new targets of known drugs (drug repositioning) and to deorphanize ligands without known targets. Previously, we presented a new method for target prediction based on the three-dimensional comparison of protein-ligand binding sites ("pockets"). Pockets are represented by clouds of points as the Cartesian coordinates of atoms lining the protein cavities. These pockets are then compared by a sequence order-independent clique-matching algorithm. Finally, a pocket similarity score is calculated based on the number of aligned atoms and their root mean squared distance. Here, we present an improved version of our method. We performed extensive validation studies against two publicly available datasets of protein-ligand complexes ("Homogeneous set" and "Kahraman set") by means of receiver operating characteristic (ROC). The performance of identifying pockets binding the same ligand was improved by 12% on the Homogeneous set. We further compared our method with the state-of-the-art algorithm IsoMIF. Our method obtained AUC values of 0.76 and 0.81 for the Homogeneous and the Kahraman set, respectively, while IsoMIF achieved values of 0.80 and 0.85, respectively. When applying optimal parameters per ligand class, we improved AUC values to 0.83 and 0.86. Automatic selection of optimal parameters is planned as a future improvement of the method. In summary, we present a fast, sequence order-independent clique-matching approach for the comparison of protein pockets with straightforward application in small molecule target prediction.

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8) Surprising folding features of the metamorphic C-terminal domain of the bacterial virulence factor RfaH.

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Gene expression is controlled by accessory factors that aid RNA polymerase (RNAP) in increasing its processivity and coupling its activity to translation. The metamorphic protein RfaH is a member of the universally conserved NusG family of transcription factors possessing an N-terminal domain (NTD) that binds to RNAP, but its C-terminal domain (CTD) is folded as an α-helical hairpin that occludes the RNAP-binding site of NTD. Upon being recruited by RNAP stalled at an ops DNA sequence, both domains are separated, the NTD binds to RNAP, and the CTD refolds into a β-barrel typical of NusG proteins that can bind the ribosome.

Although the transformation is essential for its biological function, its molecular mechanism remains unknown. Here we provide insights of this mechanism by analyzing the folding of the isolated CTD to determine its conformational stability. Circular dichroism showed the characteristic spectrum for β-rich proteins. Unfolding experiments with chaotropic agents showed a three-state mechanism. Interestingly, thermal unfolding showed an increase in dichroic signal near 200 nm, without changes at 215 nm. Additionally we designed tryptophan substitutions in several positions to follow unfolding using fluorescence. These mutants, both with exposed and buried tryptophans, showed similar stability between them and overlapped with the first transition observed for CTD unfolding through circular dichroism. These results suggest an heterogeneous folding landscape due to the metamorphic character of this protein, which will allow us to determine the energetic cost of the α-to-β transformation of the CTD in the context of the full-length RfaH.

FONDECYT 11140601

10) Angiotensin II increases total LC3 levels and induces autophagy in vascular smooth muscle cells.

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Vascular smooth muscle cells (VSMCs) are an essential component of vessels involved in vascular tone regulation. During hypertension, Angiotensin II (Ang II) levels are increased and triggers vascular remodeling and cardiovascular diseases. Here we evaluate the effect of Ang II on LC3 levels as a possible mechanism by which Ang II induces autophagy in VSMCs. Smooth muscle cells A7r5 from rat aorta were treated with Ang II (100 nM) for 24 h. LC3 were evaluated by LC3I/GAPDH, LC3II/GAPDH. LC3II+LC3II/GAPDH (Total LC3) levels using western blotting. Autophagy was evaluated by LC3II/GAPDH, p62, Beclin-1, Atg12–Atg5 levels by western blotting and autophagic flux in presence and absence of chloroquine 30 µM. A7r5 cells treated with Ang II 100 nM for 24 h showed an increase in LC3I, LC3II and LC3 total protein levels. In these conditions, an increase in autophagic flux was also observed. p62, a protein degraded by autophagy, also showed a decrease after treatment with Ang II. Two proteins involved in early steps of autophagy, Beclin-1 and Atg12–Atg5, were increased after stimulation with Ang II 100 nM. Taken together, these results suggest that Ang II induce autophagy in VSMC, probably due to an increase in LC3 total levels. This process could play a key role in the mechanism which Ang II induces autophagy in VSMCs.

FONDECYT 1140329, FONDAP 15130011.

12) Epigenetic modifications by Polycomb Group Proteins (PcG) on senescence-associated genes Ink4a/ Arf/Ink4b and Cdkn1a in hippocampal neurons.

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The epigenetic is the study of gene expression changes without DNA sequence modifications. The epigenetic mechanisms include histone post-translational modifications as methylation and ubiquitylation. All of the above can be performed by Polycomb Group Proteins (PcGs). For replicative cells, the senescence process is the arrest at the G1 stage of cell cycle to prevent an excessive proliferation. Senescence-associated proteins $p14^{Arf}$, $p16^{Ink4a}$ y $p21^{Cip}$ are coded in the *Ink4a/arf/ink4b locus* and *Cdkn1a* gene and its expression has also been described as controlled by epigenetic mechanisms mediated by PcGs. Nevertheless, is still not clear what happens in post-mitotic cells, such as neurons. We proposed here that the senescence process in hippocampal neurons is regulated by the PcGs through post-translational modifications on the senescence-associated genes, *Ink4a/arf/ink4b* and *Cdkn1a*. We described the pattern of PcGs epigenetic modifications on the *Ink4a/arf/ink4b* locus and *Cdkn1a* gene in rat hippocampus. Hippocampal tissue from rat embryos and adult, were analyzed by ChIP assays coupled to real time PCR (qPCR). Our results show that, in embryonic state, PcG proteins interact with the *Ink4a/arf/ink4b* locus and *Cdkn1a* gene inducing post-translational modifications on the H3 and H2A histones, H3K27me3 and H2A119ub. During postnatal development, when the senescence-associated genes are actively transcribed, the post-translational modifications mentioned above decreases, together with an increase in the presence of the H3K27me3 demethylases JMJD3 and UTX on the genes. In summary, our results suggest that the hippocampal neurons go through the senescence process, which can be regulated by Polycomb Group Proteins and its epigenetic modifications.

FONDECYT 11130584 (B.H), FONDECYT 11130667 (MC.V), FONDAP 15090007 (M.M) and FONDECYT 1170878 (M.M)

14) Synonymous mutations in the 3-phosphoglycerate kinase gene in Schizosaccharomyces pombe alter protein expression and cell growth.

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Folding of many proteins is a co-translational process, where the exit speed of the nascent peptide from the ribosome is critical. This process might be controlled by translation efficiency of the mRNA. Due to the redundancy of the genetic code, it is believed that codon bias is a relevant factor that control translation rate. So far, the rules that govern translational rate by synonymous codons and the effects on cell fitness are unknown. The purpose of this work was to study the effect of synonymous mutations in the gene encoding the highly expressed enzyme 3-phosphoglycerate kinase 1 (Pgk1) on protein levels and folding as well as on cell fitness of *S. pombe*. To study the effect of synonymous mutations, eleven segments of pgk1 were randomly mutated for synonymous codons and each variant was introduced in the chromosome by homologous recombination. Then, we measured cell growth in minimal medium and protein aggregation. We determined that mutations in the majority of pgk1 gene regions altered yeast growth. A concomitant alteration of protein aggregation was observed, suggesting a role of these codons in the modulation of translation and folding. These data suggest that codon usage bias is an important regulator of proteostasis.

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16) TDP-43 aggregation affects Chaperone Mediated Autophagy (CMA) pathway.

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Introduction: TDP-43 is a ribonuclear protein involves in RNA metabolism. TDP-43 protein accumulations occur in neurodegenerative diseases like ALS and FTD. Although normally localized in the nuclei, under pathological conditions, TDP-43 is observed in cell cytoplasm forming aggregates. TDP-43 has a KFERQ like-motif, which might be recognized by protein chaperone Hsc70, involved in Chaperone-Mediated-Autophagy (CMA). In this work, we study the relationship between the aggregation of TDP-43 and CMA.

Methodology: Three cell lines stably transfected were used: HEK293-Flp-in (control cell line), HEK293-TDP-43WT (TDP-43WT overexpression) and HEK293-TDP-agg (overexpression of TDP-43 aggregated form). Overexpression of TDP-43WT and TDP-agg was induced with tetracycline and CMA response was assayed by evaluating the expression of its main components (Hsc70 and LAMP-2A) through qPCR and Western blot. Relative activity of CMA and interaction of Hsc70 with TDP-agg were assayed by immunofluorescence and immunoprecipitation, respectively. As positive control, CMA activation was induced through serum deprivation (starvation).

Results: TDP-43 aggregation increases the expression of LAMP-2A and Hsc70. However, overexpression of TDP-43WT does not affect the levels of these components. In contrast to TDP-43WT, TDP-agg interacts with Hsc70 chaperone under normal and starvation conditions. Preliminary results indicate that CMA activity is inhibited under TDP-43 aggregation as observed by a decrease in the number of perinuclear lysosomes positive for LAMP-2A.

Conclusions: TDP-43 aggregates induce an increase in the main components of CMA pathway probably to overcome the TDP-43 aggregation condition or as compensatory mechanism able to mitigate an inhibited CMA activity. Results strongly suggest that TDP-43 aggregation disturbs CMA activation.

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18) Host - microbiota interactions: searching for immune response biomarkers in the scallop Argopecten purpuratus.

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The scallop *Argopecten purpuratus* is a mollusk with significant impact in Chilean aquaculture. Nevertheless, its production has declined due to disease outbreaks. It is well recognized that the associated microbiota in vertebrates protects against competing microorganisms and regulates host immunity. Indeed, fluctuations on microbiota structure could affect the immune function of organisms, increasing the probability of succumbing to diseases. In this context, host-microbiota interactions should be considered also in invertebrates when designing control strategies. Scallops are filter feeders which have developed innate immune mechanisms to maintain homeostasis and defend themselves. Therefore, we aimed to characterize the expression of *A. purpuratus* immune genes after an immune challenge and to determine shifts in microbiota composition that may be valuable as diagnostic biomarkers. For this, the transcript expression of several immune regulators and effectors was assessed from naïve and immune challenged scallops by RT-qPCR, and the structure of bacterial communities was analyzed by denaturing gradient gel electrophoresis (DGGE). Results showed that the transcript levels of several immune genes were significantly upregulated in scallop hemocytes after injection with the heat- killed bacteria *Vibrio splendidus*. In addition, dendrogram construction from DGGE profiles evidenced clearly distinct bacterial communities among naïve and immune challenged scallops. Also, the bacterial community from immune challenged scallops showed decreased richness and diversity. Overall, the activation of the immune response in *A. purpuratus* generates highly defined changes in the associated microbiota, and the identification of fluctuating bacterial species is now required to assess their function in scallop defense reactions.

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20) Identifying miRNAs and their mRNA targets in the altiplano fish Orestias ascotanensis.

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Living organisms are constantly exposed to a wide range of environmental variations that generate selective demands on them. *Orestias ascotanensis* is a wild teleost fish that habitats in extreme environments of the Chilean altiplano. Therefore, this animal has unique characteristics that make it an attractive model to address mechanisms that control gene expression in response to extreme environmental changes. Among the mechanisms that regulate gene expression in metazoans, those mediated by microRNAs have been shown to have a critical role. Nevertheless, little is known about the cell pathways and mRNA targets that are modulated by microRNAs in aquatic species, in particular living under stressful conditions. Our FONDAP center has recently sequenced at high coverage, the complete genome of *Orestias ascotanensis*, hence generating a valuable tool to begin addressing the genetic and epigenetic components that provide this fish with the ability to rapidly adapt to changing environmental conditions. Here, total RNA from wild *Orestias* was isolated and libraries were constructed for Illumina microRNA and mRNA sequencing. The resulting reads were analyzed through a bioinformatics pipeline that gave rise to 505 putative miRNAs in this fish. We identified targets for these miRNAs at the 3`UTRs of the total transcriptome, obtaining a series of miRNA/Targets. Interestingly, we found a significant enrichment of ontology terms related with control of gene transcription. Our results provide evidence supporting that miRNAs may be critical components in adaptive responses of fishes exposed to extreme environmental conditions through rapidly modifying gene expression profiles.

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22) Atorvastatin inhibits basal autophagy in skeletal muscle cells.

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Autophagy is a mechanism for protein and intracellular organelles degradation. Inhibition of autophagy, i.e. by antimalarial drugs such as chloroquine, induces different myopathies, because the accumulation of damaged proteins and organelles. Atorvastatin, a cholesterol synthesis inhibitor, also produces muscle damage by rhabdomyolysis as its main side effect. However, the mechanism by which this muscular damage is produced remains unexplored. Rat skeletal muscle cell line L6 differentiated in myotubes were stimulated with Atorvastatin 10 uM. Autophagy was determined by measuring LC3-II and p62 protein levels. Furthermore, to determine the autophagic flux, autophagy was chemical inhibited with Bafilomycin A1, an inhibitor of the lysosomeautophagosome fusion. Atorvastatin induced an increase in LC3-II protein levels from 1 to 24 h of treatment. This increase is comparable to those induced by nutrient deprivation with EBSS. However, atorvastatin did not change p62 levels unlike EBSS that significantly decreased p62 levels. On the other hand, atorvastatin did not induce a greater accumulation of LC3II in the myotubes when treated with Bafilomycin A1, as opposed to EBSS. In summary, our data suggest that atorvastatin is inhibitor of autophagy by blocking autophagy flux.

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24) Comparative Genomics of Piscirickettsia salmonis reveals structural genomic difference within genogroups.

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Introduction: *Piscirickettsia salmonis* is the etiological agent ofpiscirickettsiosis, disease that affects the salmonid industry. Despite efforts to functionally and genomically characterize *P. salmonis*, information on the pathogenesis, diagnosis and control of this fish pathogen remain lacking.

Methodology: Genomic information was obtained from NCBI, gene prediction through GLIMMER 3.02 and TransDecoder and the functional annotation through DIAMOND against GO database. The pan-genome it was obtained by GET_HOMOLOGUES software. For the phylogenetic tree were used 7 constitutive genes. For phylogenomics tree were used all core-genes. Finally, the ribosomal operons were identified by RNAmmer and gene cassette dot/icm T4SS through manual search.

Result: Through the comparison of genetic information from 19 complete genomes of *P. salmonis* was achieved the identification of open pan-genome. The strains were isolated from different hosts (*S. salar* 68.4%; *O. kisutch* 15.8%; *O. mykiss* 15.8%) from Chilean salmon industry from 1989 to 2015. The existence of 2 genogroups was confirmed through phylogenetic and phylogenetic relationships, associated to LF-89 and EM-90 type strains. In addition, the variation in the functional annotations in components of pan-genome and the rearrangement in the genomes structure (ribosomal operons and cassette T4SS), confirm the different genogroup.

Conclusions: To the best of our knowledge, this study is the most comprehensive comparative genomics characterization of complete genome for *P. salmonis*. The characterized divergences and similarities represent a contribution towards understanding the biology and evolution of *P. salmonis*. Bioinformatic analysis provide an idea of the relationship between different genogroups, corroborated by functionallity and rearrangement of genomics components.

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26) Angiotensin-(1-9) prevents Norepinephrine-Induced Cardiomyocyte Hypertrophy by controlling Mitochondrial dynamics

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Introduction: Angiotensin-(1-9) is a novel peptide of the non-canonical renin-angiotensin system. The counterregulatory action of angiotensin-(1-9) on the pro hypertrofic effects of angiotensin II position it as potential new alternative for the treatment of cardiovascular diseases with a antihypertrophic cardiomyocyte effects. The aim of this study was to examine the effect of angiotensin-(1-9) on mitochondrial morphology in the setting of norepinephrine-induced hypertrophic growth.and its stability in human serum.

Material and methods: Primary cell cultures were incubated with or without angiotensin-(1-9) and/or NE (to induce hypertrophy). Cardiomyocyte hypertrophy was evaluated with phalloidin–rhodamine and mitochondrial morphology analysis with Mitotracker green then both was observed using a confocal microscope. Mitochondrial mass was evaluated by quantifying the abundance of the constitutive mitochondrial protein mtHsp70. The serum stability was analyzed by measurement by HPLC.

Results: NE dependent declines in the average size of individual mitochondria was also prevented the double staining revealed that NE-treated cardiomyocytes exhibited a hypertrophic phenotype, determined as increases in both sarcomeric structure and cellular area together with a decrease in the relative mitochondrial area compared to controls. Angiotensin-(1-9) inhibited mitochondrial fission in cardiomyocytes treated with NE as assessed by both the relative number of mitochondrial per cell and the relative mitochondrial volume. The stability of the peptide shows that it has a considerable presence of peptide up to 5 min of incubation in serum.

Conclusion: Angiotensin-(1-9) prevented mitochondrial fission and hypertrophic growth in response to NE and exhibit normal stability for a serum peptide.

28) Glucagon-like peptide-I inhibits basal and induced autophagy via protein kinase A in vascular

smooth muscle cells.

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Introduction: Deregulation in both autophagic flux and vascular smooth muscle cell (VSMC) dedifferentiation have been associated with different cardiovascular diseases. When glucagon-like peptide-1 (GLP-1) analogs have been used in diabetes type 2 treatment, a decrease in the incidence of cardiovascular diseases has been observed. The binding of GLP-1 and its receptor (GLP-1R) activates protein kinase A (PKA), which have been described as a negative autophagy regulator. We propose that GLP-1 inhibits autophagy via PKA in VSMC.

Methodology: Aortic VSMC cell line A7r5 were incubated with either medium + 10% serum or with glucose deprivation by using RPMI medium. A7r5 were treated with GLP-1 (100nM) and H89 (100 nM, PKA inhibitor) during 4 h. Autophagic flux was evaluated with chloroquine (30μM). Autophagy was assessed by LC3 I, LC3 II and p62 levels by western blot. VSMCs were transduced by AdGFP-LC3 (MOI=180) for 24 h for the visualization of autophagosome formation.

Results: Decreased LC3 II levels were observed when VSMC were treated with GLP-1. These levels were recovered when cells were preincubated with H89. The same results were obtained when determining autophagy flux. Autophagosome formation using LC3-GFP and fluorescence microscopy showed similar results to those obtained with LC3-II.

Conclusions: Our results showed that GLP-1, through a PKA dependent mechanism, inhibits basal autophagy and glucose deprivation-induced autophagy in VSMCs. GLP-1-dependent autophagy inhibition could be a new therapeutic effect of incretins.

FONDECYT 1140329, FONDAP 15130011

30) Heterogeneous Nuclear Ribonucleoprotein K (hnRNPK) acts as an IRES trans-acting factors (ITAF) for the HIV-1, HTLV-1 and MMTV IRESs.

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The human immunodeficiency virus type 1 (HIV-1), human T-cell lymphotropic virus type 1 (HTLV-1), and mouse mammary tumor virus (MMTV) full-length mRNAs can initiate translation using an internal ribosome entry site (IRES). Some IRESs require cellular proteins, IRES trans-acting factors (ITAFs), for their function. In this study, we evaluated the role played by the heterogeneous Nuclear Ribonucleoprotein K on the IRES-mediated translation initiation using siRNA based knockdown and plasmid based overexpression assays in different cell lines. Together, results from the knockdown and overexpression assays show that hnRNPK acts as an ITAF for the HIV-1, MMTV and HTLV-1 IRESs up-regulating their translational activity. Considering that in cells hnRNPK is mainly located in the nucleus we evaluated the cellular distribution of hnRNPK in HIV-1-infected cells. Consistent with a role in translation, localization of hnRNPK is altered in HIV-1-infected cells delocalizing from the nucleus to the cytoplasm. This observation suggests that during replication HIV-1 promotes the redistribution of nuclear proteins required for IRES-mediated translation to the cell cytoplasm. This strategy would ensure the adequate expression of viral structural proteins in cells infected with HIV-1 under conditions that hinder cap-dependent translation initiation.

32) Computational study of the adsorption of some residues and small motifs from IgG on mica surface using molecular dynamics.

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Adsorption is a physicochemical interface phenomena where particles interact over a surface inducing structural and functional changes. In case of proteins, these changes affect their spatial configuration and stability. Due to adsorption relevance in many biological and biotechnological processes, it is crucial to know wich physicochemical factors are involved which are still unclear at atomic resolution.

Comprehend the adsorption process of antibodies represents an imperative challenge to face not only for its importance in biological systems but also in experimentals techniques. For Intance, immunoassays are a set of antibody-based detecting methods for antigens, whose recognition is determined by specifics paratope tags onto the immunoglobulin (Ig) variable regions. Immunoassays can be used for differents purpouses such as design of biosensors, medical diagnostics, proteomics, pharmaceutical drug screening research, etc.

In order to dilucidate the factors involved in the first steps of IgG1 adsorption, a computational simulation was performed to study the interactions between a lysine and a mica muscovita surface. The molecular dynamics was developed using ReaxFF, a program for modeling chemical reactions with atomistic potentials. Afterwards, the ΔG of the system was calculated, knowing that molecular dynamics simulations coupled with free-energy calculation techniques could predict adsorption affinities in good agreement with experiment.

The protocol here shown represents a novel insight into the adsorption mechanism of IgG1 on inorganics surface. This knowledge can be used to understand experimental results obtained by AFM imaging, a technique used in our laboratory for proteins and protein complexes structure characterization.

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34) Differential Expression of Transcription Factors involved in Epithelial to Mesenchymal Transition in Relation to Pathways Activated by TGF-β in Breast Cancer Tumors.

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Metastasis is the main cause of death among women with breast cancer. Patients with triple negative breast cancer tumors develop distant metastases earlier compared to luminal tumors. Epithelial-mesenchymal transition (EMT), induced by TGFβ signaling pathways, is the main mechanism to promote metastasis,through the expression of transcription factors TWIST, SNAIL, SLUG and ZEB1. The expression of transcription factors has been studied in cancer cell lines but no studies have been reported in breast cancer tumors. In this study we analyzed by immunohistochemistry the expression of four transcription factors as well as the state of pathways: TGFβ/SMAD, ERK/MAPK and PI3K/AKT in 100 breast cancer tumors. The same analysis was done in breast cancer cell lines: HCC1937 (triple negative) and T47D (luminal) treated with TGFβ. At least one transcription factor was expressed in all triple negative tumors (n=20), and in 66% of luminal tumors. Luminal tumors had the PI3K/AKT pathway active and showed that ZEB1 is the most frequently expressed (n=25). Triple negative tumors showed an active state of the TGFβ/SMAD pathway with expression of all transcription factors, whereas tumors with an active state of ERK/MAPK (n=5) pathway showed only ZEB1 expression. T47D cells showed activation of PI3K/AKT and ERK/MAPK pathways, and expression of SNAIL and SLUG, whereas HCC1937 cells showed activation of TGFβ/SMAD and ERK/MAPK, and expression of TWIST, SLUG and ZEB1. In conclusion, we found a differential activation of signaling pathways and transcription factor's expression, suggesting a diverse EMT mechanism induced by TGFβ, in the different breast cancer subtypes.

36) Galectin-3 promotes a paracrine communication between fiboblasts and cardiomyocytes.

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Introduction. Cardiac remodeling is characterized by cardiac hypertrophy, loss of contractility of cardiomyocytes and activation of myofibroblasts. Galectin-3 (Gal-3) is a β-galactoside-binding protein used as a prognostic biomarker in heart failure (CHF) patients. Genetic and pharmacologic studies show that Gal-3 is required for cardiac remodeling in animal models of CHF, suggesting a role in progression of this disease. However, the mechanism involved is unknown. Here we asked if Gal-3 induces cardiac remodeling and hypertrophy through a mechanism involving paracrine communication.

Methodology. Primary cardiomyocytes and fibroblasts were stimulated with Gal-3 10ug/ml and N-Acetyl-D-Lactosamine 1ug/ul to evaluate: Cell death by flow cytometry, cell proliferation by MTT assay, the activation of signaling pathways by western blot, the mRNAs: Tgfb1, Acta2, Col1a1, Myh7 and Nppa by RT-qPCR. Finally, we evaluated a panel of miRNA related to cardiovascular diseases.

Results. Our results showed that Gal-3 has no effect on cardiomyocytes. However, in cardiac fibroblast, induced ERK and AKT phosphorylation, as well as proliferation. Also, Gal-3 increases the expression of the cytokine TGF-β1, a regulator of cardiac remodeling, and miR-155, miR-199b and miR-106b. Conditioned medium from Gal-3 stimulated fibroblasts induced expression of cardiac hypertrophy markers in NRVM.

Conclusions. Gal-3 activates pro-survival and proliferation signaling pathways in cardiac fibroblasts without a direct effect on cardiomyocytes. However, Gal-3 can induce cardiomyocyte hypertrophy by a mechanism involving paracrine communication between fibroblasts and myocytes, which likely includes TGF-β1 and the miRNAs-155, -199b and -106b.

FONDAP 15130011 (PC), FONDECYT 11140470 (CQ), FONDECYT 1150359 (HV), FONDECYT 1141198 (PC), FONDECYT 3160287 (MB)

38) Synthesis of Structured Triacylglycerides with Thermomyces lanuginosus lipase in hexane and supercritical carbon dioxide (SCCO2): comparison of both methods.

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EPA and DHA polyunsaturated fatty acids (PUFA) are beneficial in human health. Their sources for consumption are fatty fishes. The use of n-3PUFA as supplements has prompted the development of structured triacylglycerols (TGs) with lipases. Supercritical carbon dioxide ($SCCO_2$), with low masstransfer resistance and high diffusivity has beenused for these lipases reactions.

The objective of this work was to synthesize TGs, with EPA or DHA and caprylic acid (CA) in its structure catalyzed by *Thermomyces lanuginosus* lipase (TL IM), in hexane and $SCCO_2$. For this, EPA and DHA of rainbow trout "belly" was concentrated by inclusion with urea. The synthesis of TG from glycerol, AC and EPA and DHA concentrate, was initially performed in hexane with the objective of evaluating the behavior of lipase TL IM (at 10% and 50% with respect to substrates) in the time (24, 48 and 72 h). TGs were then synthesized for 28 h in $SCCO_2$ with 10% TL IM lipase. The samples were analyzed by thin layer chromatography (TLC) and gas chromatography (GC). Subsequently, a response surface method (RSM) design was performed for the synthesis in hexane, using as time, temperature and agitation reaction variables in a $2^{3\ +star}$ design. The synthesis of TGs in $SCCO_2$ was optimized by RSM considering the proportion n-3PUFA:CA, percentage of glycerol, time, temperature and pressure in $SCCO_2$, through a $2^{5-1\ +\ star}$ design. The results obtained indicate that $SCCO_2$ is a more efficient means for the synthesis with the lipase of TGs with biological value.

40) Role of polycystin-1 in heart failure development and BIN1 regulation.

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Heart failure (HF) is the inability of the heart to pump the adequate amount of blood to the tissues. An impaired formation of the T-tubule in the cardiomyocyte decrease the cardiac contractility and precedes the HF. BIN1 is a crucial protein in the formation of T-tubules and its expression decrease during HF, but the involved mechanism is unknown. Polycystin-1 (PC1) is a crucial mechanosensor that maintains the heart contractility. Young PC1 *knockout* (PC1 KO) mice show an impaired cardiac function but the mechanism is unknown. We hypothesized that absence of PC1 in the cardiomyocytes induces HF development with a decreased expression of BIN1.

Methods: We assessed the survival of PC1 KO and control mice. Morphometric, biochemical (qRT-PCR) parameters and cardiac function (echocardiography) were determined. BIN1 protein content and mRNA were measured by western blot and qRT-PCR in cardiac tissue respectively.

Results: PC1 KO mice die suddenly at 8 months of age in average. Morphometric (ratio of heart weight/body weight and lung wet weight/body weight) and biochemical parameters of HF (mRNA of β-MHC, ANP, BNP) were increased in PC1 KO mice respect to the control. PC1 KO mice present a decreased ejection and shortening fraction. On the other hand, BIN1 protein and mRNA also decrease in cardiac tissue of PC1 KO mice.

Conclusion: Our results indicate that PC1 is involved in HF development and play a pivotal role as a new regulator of BIN1 expression in cardiomyocytes.

Grant support: Fondecyt 1150887 (ZP), 3160549 (PA) and FONDAP ACCDiS 15130011 to ZP and PA.

42) microRNAs targeting EMT Transcription Factors in breast cancer.

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Metastasis is the leading cause of cancer-associated deaths worldwide, promoted by transcription factors SNAIL, SLUG, ZEB and TWIST trough epithelial-mesenchymal transition (EMT). MicroRNAs are small non-coding RNAs, whose expression has been demonstrated to be altered in cancer. Because of 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.The aim of this study is to identify microRNAs downregulated in breast tumors expressing EMT-transcription factors, and that are involved in epithelial-mesenchymal transition. For this purpose, we used microRNA microarray data from 50 fresh frozen breast tumors, 28 from patients with lymph node metastasis. Transcription factor expression was measured by immunohistochemistry. Microarray data analysis using RankProd (R package) revealed approximately 40 microRNAs down-regulated in breast tumors with expression of EMT-transcription factors ($p<0,05$). By in silico analysis, we identified few microRNAs from this group predicted as regulators of SNAIL, SLUG and TWIST, and selected four: miR-202, miR-210, miR-331 and miR-34b. We evaluated by luciferase reporter assay the regulation of the 3´end of selected transcription factors by each microRNA.We found that miR-210 caused a decrease in luciferase activity trough SNAIL and SLUG 3'UTRs in 30% and 60%, respectively, miR-331 a 40% through SLUG 3'UTR, and miR-34b a 55% using TWIST 3'UTR. Our results suggest that downregulation of an important proportion of the identified microRNAs, may lead to an overexpression of their target transcription factors in primary breast tumors, inducing a metastatic behavior of tumor cells.

44) Distribución de los polimorfismos RS1801131 Y RS1801133 del gen de metilentetetrahidrofolato reductasa (mthrf) en individuos de ambos sexo de la región de Antofagasta.

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Los estudios de marcadores genéticos son utilizados para evaluar la asociación entre la presentación de una variante alélica y la susceptibilidad a desarrollar enfermedades crónicas. Entre ellos, la enzima metilentetrahidrofolato reductasa (MTHFR), que realiza la conversión de homocisteína a metionina, presenta variantes genéticas asociadas con una alteración de la función de la enzima, resultando en niveles elevados de homocisteína, que pueden aumentar el riesgo de desarrollar enfermedades cardio y cerebrovasculares.

El objetivo del presente estudio fue evaluar la distribución genotípica y frecuencia alélica para los polimorfismos rs1801131 (1298 A>C) y rs1801133 (677C>T) del gen MTHFR y su efecto sobre los perfiles lipídicos y niveles de homocisteína en una población normotensa del norte de Chile.

Para determinar la frecuencia de las variantes rs1801131 y rs1801133 del gen (MTHRF), muestras de sangre de 120 individuos adultos sanos de ambos sexos fueron procesadas para obtener ADN genómico mediante el método de precipitación salina y plasma para la determinación de Homocisteína, Colesterol total, Triglicéridos, HDL y LDL. El análisis de los polimorfismos fue realizado mediante discriminación alélica utilizando sondas Taqman y para el perfil lipídico y homocisteína mediante pruebas enzimáticas colorimétricas.

La distribución genotípica para los polimorfismos rs1801131 (1298 A>C)y rs1801133 (677C>T) del gen MTHRF mostraron una frecuencia para el alelo mutado C de 0.26 ($p < 0.01$) y para el alelo T de 0,51 ($p < 0.28$), respectivamente. Nuestros datos muestran que la variante genética no afecta los parámetros clínicos en los sujetos estudiados.

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

46) Study of the forces involved in the conformational changes associated to the ligand binding and catalysis in Adenylate kinase.

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One of the main goals in Biophysics is understanding protein structure-function relationship, because it still remains unknown how enzymes achieve high catalytic efficiency coupling catalysis with structural dynamics. A modern approach to study these events is considering them as mechanical processes, where forces, distances and energies can be determined to characterize them. Adenylate kinase (AK) is our model to quantitatively asses the importance of the strain-induced theory and to study the cracking phenomena (local unfolding/refolding event during catalysis). *In singulo* pulling force studies performed by Optical Tweezers (OT) manipulation, showed that AK unfolds reversible around 20 pN and has a length of 5 nm, with a $\Delta G_{unfolding}$ of about -4.5 kcal/mol. Simultaneously, we also performed mutations of the Val117 and Leu162 residues to Gly, considering that it has been reported that they promoted a selectively unfolding of the entire ATP lid without unfolding any other AK domain. *In silico* pulling force studies using Steered Molecular Dynamics (SMD) showed that the ATP lid is the first region to unfold at a distance of about 5.3 nm, and that the mutation in residue Leu162 has a greater effect than in the residue Val117. Our results indicate that the unfolded region seen in OT AK pulling could be the ATP Lid, because these results correlate with SMD pulling data. As work in progress, we will test the Val117Gly and Leu162Gly AK mutants in OT.

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48) Copper complex induces apoptosis in gastric cancer ags cells.

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Introduction: The high occurrence of gastric cancer has led to the development of new therapies using inorganic compounds which demonstrate better anticancer activity and diminished side effects. Transition metals have long been used to cure different diseases. One of the characteristics that metals possess is the ability to transfer electrons. The cationic form of the metal is fundamental to its functionality as cancerous DNA and proteins are rich in electrons, thus favoring attraction of metallic compounds, leading to cell apoptosis.

In this work we show the anticancer activity of the $[Cu(tpy\text{-}phCOOCH_3)_2]Cl_2$ complex on gastric cancer AGS cells.

Methods: AGS cells were grown on 96 well plates and the copper complex was added in varying concentrations. Cellular viability was determined by MTS assay. The expression of genes pro- and anti-apoptotic was analyzed by RT-qPCR whereas the activity of caspase 3 and 7 was assessed by the Apotox-Glo assay. Annexin-V and Hoechst were used as markers of apoptosis by confocal microscopy.

Results: The observed IC50 of the copper complex in AGS cells was 4,28μM. The apoptosis was induced by the upregulation of pro-apoptotic genes, and the expression of caspases 3/7 shows a positive correlation with the copper complex.

Conclusions: These results are promising for the development of a new anti-tumor drug based on Cu, as it shows antiproliferative activity and ability to induce apoptosis by means of the overexpression of apoptotic genes and the activation of caspases 3 and 7.

CORFO 14IDL2-30087.

50) Evaluation of the participation of Polycystin-1 in the effects of Angiotensin- (1-9) on the death of cardiomyocytes in ischemia / reperfusion in an in vitro mode.

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Angiotensin (1-9) is a member peptide of the Renin-Angiotensin System “Not canonical” with beneficial functions on the cardiovascular system, these include reducing cardiovascular remodeling, having antihypertensive and cardioprotective functions since it prevents cardiomyocyte death due to ischemia and / or reperfusion. Its actions are through the angiotensin receptor type 2, however, it is required to know more in depth the mechanism of action of angiotensin- (1-9) to effectively carry out a new pharmacological agent with cardiovascular effects.

In the search to expand the cardioprotective pharmacological knowledge of Ang- (1-9), in this we set out to study if Polycystin-1 an integral protein of the membrane with the cardioprotective activity and with the capacity to regulate other proteins, is capable of favoring this protective effect.

In an in vitro model of simulated ischemia and subsequent reperfusion, a culture of ventricular cardiomyocytes of neonatal rats, the expression of Polycystin-1 wasdiminished, and various markers of cell death were evaluated. The results showed that the cardioprotective effect on ischemia / reperfusion of Ang- (1-9) was lost when Polycystin-1 expression is decreased.

FONDAP 15130011 Centro avanzado de enfermedades crónicas “ACCDiS”

52) Linking the fold-switching behavior and the transcriptional activity of the metamorphic bacterial virulence factor RfaH.

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RfaH is a bacterial virulence factor responsible of promoting transcriptional elongation and ribosomal recognition of otherwise low-expressing genes. This function is exerted through its N-terminal domain (NTD), which binds to RNA polymerase (RNAP) upon recruitment by a pause-inducing sequence termed *ops* to increase transcriptional processivity; and its C-terminal domain (CTD), which is bound to the RNAP-binding interface of the NTD as an α-helical hairpin to prevent spontaneous recruitment and undergoes a dissociation-induced structural transformation into a β-barrel that enables ribosomal recruitment. Since domain dissociation within RfaH is essential for RNAP binding, we assessed the influence of CTD chain flexibility in promoting transcription. RfaH backbone flexibility was evaluated by hydrogen-deuterium mass spectrometry, resulting in high anisotropy of secondary structure stability in its α-fold. Deuterium incorporation in the "tip" of the hairpin is below 30%, suggesting low backbone flexibility, whereas the ends of each helix reach up to 56% deuterium exchange. With this information, two single-point alanine mutations at both sites were evaluated by *in vitro* single-round transcription assays of RNAP stalled in *ops*-containing or *ops*-scrambled templates. While a F123A mutation in the highly flexible site mostly resembles the behavior of wild-type RfaH in both scenarios, an equivalent F130A mutation located in low-flexibility regions within the "tip" of the hairpin greatly increases *ops*-independent transcriptional activity, suggesting spontaneous recruitment to the transcriptional machinery. This relationship between backbone flexibility and anti-pause activity is highly correlated with previous *in silico* results and allows connecting the biophysical properties of RfaH metamorphosis with its biological function.

54) Characterization of Mn2+ binding site in agmatinase like protein (ALP).

Reyes M B[1], Navarrete C[1], Mardones E[1,2], García D[1,2], Mella K[1,2], Arriagada L[1,2], Martínez J[3,4], Carvajal N[1,2], Uribe E A[1,2], [1]Laboratorio Enzimología, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas , Universidad de Concepción.[2]Laboratorio Enzimología, Departamento. Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.[3]Laboratorio Biofisica Molecular, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas , Universidad de Concepción.[4]Laboratorio Biofísica Molecular, Departamento. Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.

Agmatine is a polyamine that functions as a neurotransmitter, has hypoglycemic actions and produces anticonvulsant, anti-neurotoxic and antidepressant-like effects. In mammals, agmatine can be converted to putrescine and urea by agmatinase-like protein (ALP). ALP is a new type of enzyme and has been identified in human, rat and mouse brain tissues. Recombinant rat brain ALP, is the only mammalian protein that exhibits significant agmatinase activity *in vitro* and we demonstrated its ability to generate putrescine *in vivo* conditions. ALP, in spite of differing in amino acid sequence, like all members of the ureahydrolase family, it is strictly dependent on $Mn^{2+}$for catalytic activity. However, the Mn^{2+} ligand are not yet defined and any approximation to the active site is impeded by the lack of structural information and the low degree of sequence identity of ALP with all known ureohydrolases. However, we have generated a structural comparative model for ALP, from very low sequence similarity between ALP and the template of crystals structures of prokaryotic agmatinases. The sequence of the ALP protein contains numerous variations in the predicted Mn^{2+} site. Considering our model, we generated two ALP variants: ALP-E286A/K288A and ALP-N205A/ Q213A/K288A. In the first case, the variant show a minor value of K_m for agmatine and in second case the variant resulted inactive. These results indicate that these residues would be important in agmatinase activity of ALP and would be in the proximity of active site. In addition, these results support our structural model of Mn^{2+} binding site in ALP.

Vrid Enlace 340/17. Universidad de Concepción.

56) Microsencond Molecular Dynamics Simulations of Bipartite HTH Transcription Factors and duplex DNA suggest that both helices are required for DNA binding.

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MarA and Rob are two transcriptional activators of the AraC/XylS family found in enterobacteria, which activate the regulons marRAB/soxS/rob/micF. These monomeric proteins possess two helix-turn-helix motifs as DNA binding domains, and Rob also has an additional regulatory domain. Models derived from X-ray crystallography show that MarA interacts with the mar promoter DNA sequence using its H3 and H6 helixes involving both helix-turn-helix motifs, while Rob binds its micF promoter only with its H3 helix. Previous protein-DNA binding *in vitro* experiments in our lab have shown that swapping the DNA regions where the H3 and H6 helixes bind prevents the formation of the protein-DNA complex for MarA and Rob.

We believe that since both MarA and Rob do not bind to the DNA when both binding regions are swapped, the Rob X-ray structure does not represent the true binding mode. Using the nucleic acid force field AMBER99-BSC1 and the molecular dynamics software GROMACS, we have generated microsecond long explicit water simulations of the MarA/mar and Rob/micF protein-DNA complexes. Analysis of the protein-DNA interfaces of the simulations shows that MarA spends over 98% of the time bound to DNA with both H3 and H6 helixes, while Rob spends 71% of the time bound with both helixes, the remaining fraction is spent bound only to the H3 helix. Thus, the crystal structure for Rob does not represent the most likely binding mode. Future SAXS experiments are planned to confirm our findings.

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58) Differential chromatin association of CoREST proteins.

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CoREST proteins are transcriptional co-repressors that regulate gene expression during development and in differentiated cells. They form biochemically stable protein complexes with the H3K4 demethylase LSD1 and histone deacetylases HDAC1/2 to coordinate chromatin modifying activities in one biochemical entity. CoREST proteins have different biochemical properties in terms of protein complex composition and chromatin modifying activities, leading to different repressive capacities. Several mechanisms have been described to explain how CoREST1, the most studied member of CoREST family, associates to chromatin. However, it is unknown whether CoREST2 and CoREST3 display similar capacities compared to CoREST1 or have specific association to chromatin. In this work, we hypothesized that, CoREST1, CoREST2 and CoREST3 have different chromatin association patterns. We aimed to biochemically characterize them regarding ionic strength resistance and distribution along different chromatin domains. We settled up a cellular model for neuronal differentiation, and then we performed subcellular fractionation and salt-induced extraction of chromatin proteins to compare the distribution and relative chromatin affinity for each CoREST during neuronal differentiation. We found differences that can explain how three co-repressors belonging to the same family developed different functions to accurately regulate gene expression. This work was supported by Regular FONDECYT grant number 1150200 (to MEA), FONDECYT Postdoctoral grant number 3160308 (to VN), and CONICYT fellowship for PhD. students 21161044 (to CR).

60) Purification of recombinant amyloid-β peptide and aggregation kinetic analysis by global fitting.

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Amyloid structures are created by the aggregation of amyloidogenic proteins and peptides such as amyloid-β peptide (Aβ), which are directly related to Alzheimer´s disease. Consequently, several compounds, such as antibodies, small molecules, peptides, nanoparticles, among others have been developed in order to block the formation of amyloids. Although many studies have been designed to determine the effectiveness of anti-amyloidogenic compounds, these approaches have not been suitable to determine the molecular mechanism by which a drug impairs the formation of fibers. In this work we present the purification of recombinant form of amyloidogenic Aβ, together with the experimental setup to analyze its aggregation kinetics. The experimental setup allow the application of a detailed kinetic approach to obtain information about the microscopic process underlying the amyloid formation. This approach could be used to determine the mechanism of action employed by several therapies developed to inhibit the formation of aggregates.

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62) LabNettings: scientific collaborative consumption network.

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Introduction: Science in Chile as in other countries wastes a great deal of resources, between unused and expired materials, as available equipment, which translates in a higher cost of research because of that waste. On the other hand, providers in Latin American countries take weeks to deliver orders to laboratories, delaying their research.

Methodology: Test the online platform prototype of LabNettings by showing it to lab chiefs, lab managers and investigators.

Results: We reached 82 investigators (23% Lab Chiefs, 26% Lab Managers, 51% Investigators), from 6 different universities in Santiago, Chile. 80% of the laboratories we interviewed throw away materials in a regular base, and on top of that a big part is wasted without noticing because of expiration dates. The main benefits they acknowledge are: collaboration, reduction of costs and organization. Thanks to this study we already have our first users to test the platform and 2 providers of materials willing to be part of LabNettings.

Conclusions: Access to resources and collaboration is a need in scientific laboratories. This will be solved with an online platform where each laboratory can choose what to share or exchange in order to gain access to other people's resources, and so reducing their waste of time and money in research.

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Sponsored by Dr. Christian A.M. Wilson (Universidad de Chile)

64) Development and characterization of the first lithiasis-associated gallbladder cancer model in mice.

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Gallbladder cancer (GBC) is the most common malignant tumor of the biliary tract. Chile has one of the highest GBC mortality rates. The main risk factor for GBC is the presence of cholelithiasis. Most cases follow a histological progression from metaplasia to-dysplasia and-GBC. Numerous studies have associated chronic inflammation with the development of cancer. But in GBC, there are limited studies on this topic, which present only indirect evidence due to the inaccessibility to the organ and the impossibility to follow-up the disease once the gallbladder has been removed. Nonetheless, it is accepted that chronic inflammation is the first step of metaplasia-dysplasia-GBC sequence. There are no consistent studies to confirm this hypothesis.

We have developed a murine GBC model that mimics human GBC progression from gallstone disease developed by high-fat-diet consumption. Mice were divided as control group (low-cholesterol-diet) and D-Lit group (high-fat-diet). Nine and eight D-Lit mice were euthanized at 3 and 9 month of diet. 100% of D-Lit mice developed gallstones. Further, 100% D-Lit mice (8/8) developed pseudopyloric metaplasia and 50% (4/8) developed dysplasia at 9 month-diet while at 3 month-diet only 44% developed metaplasia.

Then, we measured *ex vivo* the systemic immune cellular changes induced by each diet. We found that CD4+FoxP3+IL-10+ T cells, showed a significant increase in Treg population and also, interstitial macrophages and dendritic cells $CD103^{+}$ increased in D-Lit mice.

This is the first time that a GBC mouse model is generated from gallstone disease, where metaplasia-dysplasia-GBC can be followed.

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66) Limits of in silico target prediction of small molecules by chemical similarity.

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The identification of biological target proteins of small molecule compounds is important for polypharmacology in drug discovery and drug repositioning. Target prediction based on chemical similarity is motivated by the frequent observation that structurally similar compounds have similar physicochemical properties and possibly similar biological profiles. We have devised a straightforward method for target prediction by representing small molecules by molecular fingerprints and predicting target proteins with a nearest neighbor estimator based on the Tanimoto coefficient (Tc) of query molecules to annotated ligands of biological targets. We validated our approach with a dataset of 492,879 drug-protein interactions between 323,489 ligands and 2,161 targets, derived from ChEMBL. Results were analyzed by receiver operating characteristic (ROC), precision-recall (PR) analysis and 10-fold cross-validation obtaining excellent values for the area under the ROC curve (ROC-AUC) of 0.99 and PR-AUC of 0.64, comparable to results reported by others. However, these results were biased by a large number of similar compounds. We therefore devised a UCLUST-inspired greedy clustering algorithm to cluster molecules based on Tc similarity. Performance dropped (ROC-AUC < 0.9, PR-AUC < 0.1) with compounds clustered at Tc < 0.6. Interestingly, Silhouette analysis indicated that Tc values of 0.6 corresponded to optimal clustering. Our analysis indicates limits of current approaches based on chemical similarity, as they heavily rely on strong similarities in the dataset and are likely to fail with novel chemotypes.

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68) Physiological evidence that Piscirickettsia salmonis produce siderophore and use iron from different sources.

Ruiz P[1,4], Calquin P[3,1], Oliver C[2,1], Sanchéz P[3,1], Haro R[4], Oliva H[5], Vargas-Chacoff L[7,6], Avendaño-Herrera R[2,1,8], Yáñez A[3,1], [1]INCAR Interdisciplinary Center for Aquaculture Research .[2]Laboratorio de Patología de Organismos Acuáticos y Biotecnología Acuícola Universidad Andrés Bello.[3]Instituto de Bioquímica y Microbiología Universidad Austral de Chile.[4] Instituto de Bioquímica y Microbiología Universidad Austral de Chile.[5]Camino a Melipilla, Cerrillos Veterquímica S.A..[6]Centro Fondap de Investigación de Altas Latitudes (IDEAL) Universidad Austral de Chile.[7]Instituto de Ciencias Marinas y Limnológicas Universidad Austral de Chile.[8]Centro de Investigación Marina Quintay (CIMARQ) Universidad Andrés Bello.

Piscirickettsia salmonis is a facultative intracellular Gram-negative bacterium that causes severe economic losses in farmed salmonids in Chile. Although genome of *P. salmonis* encode genes for the iron acquisition machinery, including genes necessary for the synthesis and transport of the siderophore, no biological tests have been developed to confirm its presence. Using five isolates of *P. salmonis* and the type strain LF-89^T, we clearly demonstrate for first time the physiological production of siderophore and the capacity of this bacterium to obtain iron from different complex sources. All strains grew in the presence of the chelating agent 2,2'-dipyridyl and produced siderophores on CAS assays. Furthermore, *P. salmonis* were able to use ferric ammonium citrate and ferric nitrate as the only iron sources. In addition, *in silico* analysis identified all genes necessary for the iron acquisition systems in the *P. salmonis* AUSTRAL-005 genome. Future *in vivo* assays are needed to establish the relationship between iron uptake ability and virulence in this fish pathogen.

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70) Fast and easy labeling of extracellular vesicles with a lipophilic fluorophore for visualization and in vivo tracking.

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Introduction: The common procedure for the visualization of extracellular vesicles *in vivo* involves the labeling with fluorescents probes like PKH67 or DiR lipophilic dyes [1]. The use of density gradients are the gold standard methods for the purification of labeled-EVs but are time consuming, cumbersome and require expensive equipment [2]. Here, we established a simple and fast methodology for the obtaining of labeled-EVs for the *in vivo* tracking and visualization.

Methodology: EVs from gastric cancer cells line were incubated with DiR. For the separation of EVs-DiR of free DiR two procedures were performed; the use comercial exo-spin column, and the standard optiprep density gradient by ultracentrifugation. The eluates obtained from exo-spin column and fractions from density gradient were characterizing by size, protein content, and fluorescence intensity. EVs-DiR purificated by exo-spin method was intravenously injected in mice and visualized at 76 hour on an In Vivo FX pro imaging system.

Results: By means of exo-spin method its was obtained EVs labeled with DiR without free DiR, this was confirmed by the characterization steps. We established that column can be used several times with reproducible result and with low sample loss. The working time was just only 35 minutes compared with the at least 24 hours of the density gradient. By last, it was feasible to observe the *in vivo* biodistribution of EVs-DiR.
Conclusions: We compare both method and established that the use of exo-sin column is a procedure with reliability, reproducible, simple, fast and with no need for expensive equipment for the obtention of EVs-Dir labeled.

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72) Effect of enzymatic elimination of n-glycosylations on the structure and immunogenic properties of mollusk hemocyanins.

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Hemocyanins from mollusks *Megathura crenulata* (KLH), *Concholepas concholepas* (CCH), and *Fissurella latimarginata* (FLH) are glycoproteins used in biomedicine as non-specific immunostimulants with beneficial clinical outcomes. These effects have been attributed to their molecular weight (4-8 MDa), complex structure, xenogenicity, and carbohydrate content (approximately 3% w/w), mainly mannose-rich N-glycosylations. Hemocyanins are incorporated by antigen presenting cells and C-type lectin receptors have a role in this process, such as Mannose Receptor (MR), DC-SIGN, Dectin-1 and Dectin-2, which recognize mannose-rich N-glycosylations. However, the role of these sugar moieties in structure and immunogenicity of KLH, CCH and FLH has been poorly studied. We propose that enzymatic N-deglycosylation of these hemocyanins affect their didecameric structure and diminishes their immunogenic properties in mammals.

Hemocyanins were N-deglycosylated using PNGase F, and analyzed by dot blot using lectins and PAS staining. To compare, hemocyanins were chemically deglycosylated with sodium periodate. Analyses by SDS-PAGE and transmission electron microscopy showed N-deglycosylation of hemocyanins did not produce structural cross-linking as deglycosylation using periodate, but affects their didecameric structure. ELISA analyses demonstrated that chemically deglycosylated hemocyanins reduce their binding to MR and Dectin-2 chimeric receptors; results with N-deglycosylated hemocyanins are in progress, as well as their effect on immunogenicity. With this purpose, C57BL/6 mice were inoculated with native and enzymatically N-deglycosylated hemocyanins, and specific antibody titers in the sera of mice will be estimated. Collectively, these studies will contribute to reveal the role of N-glycosylations in the immunogenic properties of hemocyanins.

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74) Adenosine deaminase decreases chemoresistance in glioblastoma.

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Introduction: Glioblastoma multiforme (GBM) is a type of brain tumor that is highly proliferative, invasive and chemoresistant. This is mainly due to its Multiple Drug Resistance (MDR) phenotype, particularly in the glioblastoma stem-like cell (GSCs) subpopulation. GSCs express high levels of Multiple drug Resistance-associated Protein-1 (MRP1) compared to differentiated cells. Furthermore, GSCs produce high levels of extracellular Adenosine (Ado) which is involved in MRP1-mediated chemoresistance. We propose that GSC chemosensitization could be achieved by degrading adenosine with adenosine deaminase (ADA). The aim of this work was to study whether ADA decreases extracellular adenosine levels and MRP1 expression/activity in GSCs and, therefore, decrease chemoresistance.

Methodology: U87 and U87-derived GSC cell lines were treated with ADA 1U/mL for 24 h. We measured extracellular Ado by HPLC. MRP1 expression was measured by RT-qPCR and western blot, and MRP1 activity was measured after incubation with CFDA (MRP1 substrate) by flow cytometry. Cell viability was evaluated using the MTT assay by treating cells with vincristine, doxorubicin or teniposide. **Results:** we detected a reduction in extracellular Ado levels and MRP1 protein levels after treatment with ADA. An increase of CFDA accumulation was observed after treatment with ADA, indicating a decrease of MRP1 activity. Finally, we detected reduced cell viability when using a combined treatment of ADA with doxorubicin.

Conclusions: ADA reduces MRP1 activity in GSCs. Decreasing chemoresistance in glioblastoma.

76) PDGF-BB decreases mitochondrial function and induces mitophagy during VSMC phenotypic switch.

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The development of vascular pathologies such as atherosclerosis and hypertension involves the phenotypic switch of vascular smooth muscle cells (VSMC) from a contractile to a proliferative phenotype. It has been demonstrated that this process requires mitochondrial fission and autophagy. We propose that PDGF-BB, main inductor of this phenotypic switch, also promotes a decrease in mitochondrial function and induces mitophagy. Two cellular models, rat VSMC A7r5 and human smooth muscle cells (HASMCs), were used and treated with PDGF-BB 20 ng/mL. Mitochondrial function was evaluated assessing mitochondrial potential, ATP content and oxygen consumption rate. Mitophagy was determined by measuring mitochondrial mass and proteins from the canonical pathway of mitophagy, PINK1 and Parkin. PDGF-BB decreased mitochondrial potential and oxygen consumption at 24 h in A7r5. In HASMCs, these decreases were observed at 30 min and 3 h, respectively. In A7r5, PDGF-BB induced a decrease in mitochondrial DNA content a 3 h. In A7r5 and HASMC, a PDGF-BB-dependent decrease in mitochondrial protein markers was also observed. PDGF-BB also increased PINK1 content in A7r5. These data suggest that PDGF-BB induces mitophagy in A7r5 and HASMCs. A better understanding of the mechanisms underlying VSMC phenotypic switch is critical for the development of potential new treatments for vascular diseases.

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78) Separation and characterization of asparagine deamidation and aspartate isomerization using capillary electrophoresis-mass spectrometry (cesi-ms).

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This abstract describes a CESI-MS approach to monitor deamidation products in polypeptides. Nonenzymatic asparagine (Asn) deamidation and aspartate (Asp) isomerization of peptides and proteins represents an important degradation reaction occurring in-vitro in the course of isolation or storage and in-vivo during development and/ or aging of cells. Determination of modified sites in order to assess their biological significance is still problematic. A synthetic peptide mixture consisting of Asn, Asp and isoAsp containing peptides was analysed. Identification of the isoAsp containing peptide was performed via electron-transfer dissociation (ETD) fragmentation, as ETD can differentiate between the two isoforms Asp/isoAsp because of the formation of a characteristic z-57 fragment ion during the fragmentation process. However, reporter ion intensity is often very weak and not all peptides are amenable for ETD fragmentation. Therefore, a reliable separation is an important prerequisite for the analysis of deamidation products. As deamidated forms differ from their parent molecule by an additional negative charge, and slight differences exist between the pKas of Asp and isoAsp, CE enables resolution of the modified peptides. We determined the deamidation rate of histone H1.0 directly in a crude protein fraction. An assignment of amino acids in the polypeptide chain was possible due to the migration order obtained by the CE electropherogram profile. This enables to study not only the formation of Asp via deamidation of asparagine or isomerization of aspartic acid, but also to compare isoAsp levels of different peptides due to the activity of protein L-isoaspartyl methyltransferase (PIMT), an isoAsp repair enzyme.

80) Autoantibodies against fructose-1,6-bisphosphatase isolated from serum of autistic children increase intracellular ca²+ and induce ca²+ waves in primary cultures of astrocytes.

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Autism is a complex disease characterized by behavioral deficits, systemic metabolic abnormalities, frequent manifestations of lactic acidosis and the presence of serum auto-antibodies targeting key proteins in the brain of autistic patients. The aberrations of brain energy metabolism may involve mitochondrial metabolic dysfunctions within the CNS, possibly caused by a decrease in the rate of lactate utilization during gluconeogenesis. Because the levels of fructose 1,6-bisphosphatase (FBPase) and Fru-1,6-P_2 are at the core of the gluconeogenic pathway and both might be involved in the regulation of cell survival, we hypothesized their link with the neurometabolic conditions and symptoms often observed in autistic children. Using immuno-analysis techniques, we found anti-FBPase IgG in autistic patient sera that cross-react with liver and muscle FBPase (FBP1 and FBP2). We purified this autoantibody using immunoaffinity chromatographic techniques and analyzed its functions. The autoantibody does not affect FBPase enzymatic activity or its susceptibility to AMP, suggesting that the antigenic region is not in the FBP1 functional domains. Nevertheless, the possibility remains that this antibody may affect the protein to protein interaction of the FBPase isoforms with other structural or metabolic targets. Results obtained by MTT assay and immuno-active caspase 3 suggest that the anti-FBPase antibody is not harmful to the cell but increases intracellular Ca^{2+} and induces Ca^{2+} waves in primary cultures of mouse astrocytes. We conclude that autistic patients have a high level of auto-antibodies, targeting both FBPase isoforms, with undetermined metabolic and cellular effects.

FONDECYT 1141033.

82) Ancient DNA extraction from herbarium and bark-cloth samples to understand human dispersal of Broussonetia papyrifera across the Pacific

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Museum collections are prized sources of information about the past. Plant and animal collections, as well as ethnographic objects of biological origin have become recently amenable to genetic analysis following ancient DNA (aDNA) protocols, allowing the direct reconstruction of certain aspect of the past. *Broussonetia papyrifera* is a dioecious tree native of South and East Asia, which was introduced by humans to the Pacific islands as a source of fiber for the production of textiles, known as bark-cloth or *tapa*. *B. papyrifera* from Pacific islands can be used as a proxy for understanding human migratory events in the region through the study of herbaria and bark-cloth samples.

We report the successful extraction of DNA from both herbarium and bark-cloth samples, with yields between 0.5 – 5.0 and 0.1 – 0.2 µg of DNA/mg of sample, respectively. It was possible to obtain functional (amplifiable) DNA from herbarium samples that are over 100 years old and from a 250 years old bark-cloth sample from an archaeological context. DNA was analyzed with several genetic markers following aDNA protocols. The analysis of the herbarium samples using microsatellites has revealed a higher genetic diversity than in contemporary *B. papyrifera* samples, assessed through the detection of alleles and genotypes not observed in extant populations. Analysis of the aDNA extracted from the archaeological bark-cloth samples using microsatellites revealed that this piece was manufactures from more than one individual. aDNA analysis from *B. papyrifera* opens a window to the past to understand the dispersal history of this plant.

FONDECYT 1120175.

84) Evaluation of potential biomarkers of diabetic nephropathy in healthy and diabetic patients.

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One such long-term complication of diabetes is diabetic nephropathy (DN), which happens to be the leading cause of renal insufficiency in Chile and worlwide. The microalbuminuria, or the presence of proteins in the urine, is the first clinical sign of DN. The albuminuria indicates defects in the permeability of the glomerular filtration barrier; however there are a number of diabetic patients who develop ND without presenting microalbuminuria. Also, this clinical manifestation is present in advanced stages of the disease, when the morphological changes in the kidney are irreversible. For these reasons new studies in the search of early DN markers are indispensable. We investigate the protein content of urinary exosome from control and diabetics rats, with/without renal damage by LC-MS/ MS. We found 9 potential clinical biomarkers that are differentially present in diabetic samples. Subsequently, we evaluated the presence of these markers in urine of control and diabetic patients by selected reaction monitoring (SRM) for quantitative proteomics. Our results indicate that exosomes may be a useful tool for the search for new biomarkers, and specifically urinary exosomes could be used to early detection of diabetic nephropathy.

Innova-Corfo 13IDL2-23502.

86) DNA damage and repair, DNA base excision repair gene variability and risk of recurrent depression disorder.

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Background: Even though depression disorder (including the recurrent type – rDD) is common its pathogenesis still remains elusive. Elevated level of DNA damage was observed in patients with depression. Furthermore, single-nucleotide polymorphisms (SNPs) of base excision repair (BER) genes may modulate the risk of this disease. Aims: The aim of this study was to investigate the association between DNA damage, DNA repair, the presence of polymorphic variants of BER genes and occurrence of depression.

Methods: The study was conducted on peripheral blood mononuclear cells (PBMCs) of 94 patients diagnosed with depression and 119 controls without mental disorders. Comet assay was used to assess endogenous (oxidative) DNA damage and efficiency of DNA damage repair (DRE). TaqMan probes were employed to genotype 12 SNPs of BER genes.

Results: This study shows that depression is accompanied by increased oxidative stress-induced DNA damage combined with an impaired DNA damage repair efficiency. DRE was significantly higher in the controls and was modulated by BER SNPs, particularly by the c.977C>G – hOGG1, c.972G>C – MUTYH, c.2285T>C – PARP1, c.580C>T – XRCC1, c.1196A>G – XRCC1, c.444T>G – APEX1, c.-468T>G – APEX1 or c.*50C>T – LIG3.

Conclusions: We suggest that both oxidative stress and impaire DNA damage repair mechanisms contribute to elevated levels of DNA lesions observed in depression. Additionally, lower DRE can be associated with the presence of BER genes variants.

88) Allele diversity in anthocyanins synthesis genes in accessions of native Solanum tuberosum subsp. tuberosum of The Potato Genebank at the Universidad Austral de Chile.

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The potato crop has become an important resource for world food security. It has not only the purpose of human alimentation, but also functional properties due to its high antioxidants concentrations, especially by genotypes with pigmentation, specifically on the tuber pulp. The D, P and R loci are involved in the synthesis of anthocyanins and pigmentation in potato tubers. The Potato Genebank of the UACh has accessions that show pigmentation in their pulp and/or skin with high anthocyanins content. The aim of it research is to determine the allelic diversity of 96 accessions with high and low anthocyanin content for the loci D, P and R. The next generation sequencing technique (NGS), was used for genomic analysis. The results showed that for the D locus, one allelic variant was identified, as mutation present in all high anthocyanin content accessions and some accessions with low contents of these compounds. Greater allelic diversity was observed at the P locus, correlated positively with the purple pigmentation presented in most of the evaluated accessions. One accession presented a nucleotide insertion in the exon region of the analyzed amplicon, correlated positively with the high anthocyanins content and purple pigmentation in tuber skin and pulp. The R locus showed lower allelic diversity as expected because of the less accessions with red pigmentation on the Potato Genebank. This study is one-step forward in the description of native potatoes, allowing selection of high-pigmented accessions, looking forward to develop new breeding material for healthy nutrition of human being.

90) Blockade of adenosine a2b receptor in a diabetic nephropathy animal model affects leukocyte transendothelial migration molecules.

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Introduction: Progression of diabetic nephropathy (DN) is linked to pathogenic adenosine signaling through the A2B receptor subtype affecting the function of glomerular cells. The proteinuria and induction of cell fibrotic activation may be ameliorated by using an A2B receptor antagonist. Our aim was to determine through functional genomics the downstream pathways of the A2B receptor affected by the selective antagonist MRS1754.

Methodology: Experimental diabetes was induced in male rats by using streptozotocin (STZ, i.v. 60 mg/kg). Rats were treated with MRS1754, the adenosine A2B receptor antagonist (2.5 mg/kg i.p. for 1 month) or its vehicle. Kidney glomeruli were purified using a sieving method. Transcriptomic analysis by RNA-seq was carried out using the Illumine platform at AUSTRAL-Omics and performing networking by bioinformatic analysis.

Results: Differentially expressed transcripts ($P < 0.01$) were used to networking analysis. By using KEGG pathway was evidenced Cell adhesion molecules (CAMs) more significantly affected in glomeruli of the diabetic rats treated MRS1754 (*P* value 3.6 E-8). Specifically, the leukocyte transendothelial migration molecules integrin alpha 4, integrin alpha M, integrin alpha L, integrin beta 2, integrin beta 7 and Selectin P ligand were downregulated.

Conclusions: Leukocyte infiltration into glomeruli has been identified as a key event mediating diabetic glomerulopathy. We concluded MRS1754, an A2B receptor antagonist could interferers this event being able to ameliorate the renal injury in diabetic kidneys.

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92) Expression of Fructose-1,6-bisphosphatase in the differentiation process of spermatocytes into spermatids with different gluconeogenic substrates

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Spermatogenesis in adult mammals is a highly organized and regulated process that takes place in the seminiferous epithelium of the testis that supports sperm production. This process is affected by stress conditions, diabetes and biological aging, it is therefore of great importance to understand the mechanisms that control the cellular population of the seminiferous tubule. The differentiation of male germ cells has significant selection processes and metabolic changes such as: high apoptotic rate at the level of spermatocytes, adaptation to nutritional conditions, enzyme-changing expression levels and transporters related to energy metabolism. One of the enzymes involved corresponds to fructose-1,6-bisphosphatase (FBPase), the main regulated enzyme in the gluconeogenic pathway. The expression levels of protein and transcript of isoform 1 have been found increased, from spermatocytes to spermatids in testis. Furthermore, muscle isoform of FBPase (FBP2) has been described to be involved in calcium stress adaptation process by its association to the mitochondrial membrane in cardiomyocytes. Therefore, this enzyme could have a putative protector role in spermatocytes. Our main task was to determine to what extent FBPase is essential for the correct development and meiotic differentiation of male germ cells and how its expression favors the viability of spermatocytes under cytotoxic conditions. For this purpose we used mouse pachytene sperm cell line GC-2(spd), to study the differentiation of spermatocytes into spermatids and evaluate the expression of FBPase at protein and transcript level in different conditions of gluconeogenic substrates such as lactate and pyruvate, plus evaluating cellular viability under conditions of oxidative stress.

FONDECYT 1141033 (JCS)

94) Study of circulating microRNAs in heart failure with preserved ejection fraction (HFpEF).

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Introduction: Heart failure (HF) with preserved ejection fraction (HFpEF) is an increasingly recognized form of HF characterized by abnormal myocardial relaxation without overt impairment of left ventricular contractile function. Diagnosis of HFpEF is challenging since its clinical manifestations are unspecific. We assessed the usefulness of a set of microRNAs (miRs) identified by bioinformatic analysis (miR-1-3p, miR-144-3p and miR-185-5p), as potential biomarkers for HFpEF.

Methodology: Total miRs were isolated from plasma from patient with HFpEF and healthy, age-matched controls. After quantifying miR content, cDNA was synthetized using standard protocols. For specific miR identification, qPCR was performed with primers designed using miRprimer2 Software. We used mir-39 (spike-in) as a control for the extraction procedure. Hemolysis was ruled out by mir-451a/mir-23a ratio. The relative expression of each miR was determined using the ΔΔCt method.

Results: In patients with HFpEF, levels of miR-1 are significative reduced ($p<0,0001$, fold-change (FC)=0,2870). Conversely, miR-144 -3p levels are markedly increased ($p<0,0011$, FC=1,1998). miR-185-5p levels remain unaltered when compared to controls. Pathways potentially affected by this changes in miRs were assessed using Ingenuity pathway analysis, showing target genes such as Nrf2 (by miR-144-3p) and NCX (by miR-1-3p) as potential mediators of HFpEF pathogenesis.

Conclusions: HFpEF is associated to unique changes in miR levels when compared to healthy population, which may be harnessed as potential biomarkers. Furthermore, pathway analysis revealed new gene networks involved in the pathogenesis of the disease.

FONDECYT 1150359 (HV), 11140470 (CQ), FONDAP 15130011 (HV)

96) Effect of gold nanoparticles on amyloid-β peptide aggregation.

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The nanotechnology has gained a great relevance in the nanomedicine area, where efforts have concentrated in the detection, diagnostic and therapy of a specific disease. Within this area, the gold nanoparticles (GNP) have been highlighted because of their stability, biocompatibility and simple synthesis. These GNP can be functionalized with different molecules (drugs, peptides, etc) to transport them and can cross biological membranes, such as blood-brain barrier. All of these properties become them in a great tool to generate future therapeutic strategies against diseases. In our laboratory, we have extensively studied the potential use of GNP for disaggregation of amyloid-β peptide (Aβ), which is involved in Alzheimer´s disease. In the present work, gold nanoprisms (GNPr) were functionalized with D1 peptide (GNPr-D1), a beta sheet breaker, and we evaluated its effect on the Aβ aggregation kinetic. The formation of amyloid fibrils displays sigmoidal kinetics followed by fluorescence of thioflavin T. The fluorescence of thioflavin T is proportional to the formation of beta structure induced during Aβ aggregation. Our results show the Aβ aggregation kinetic has a secondary nucleation process as indicated by the gamma value to -1.435. The presence of GNPr-D1 during the Aβ aggregation decreases the gamma value to -2.095. This value indicates the secondary nucleation process is not affected by GNPr-D1, although the presence of GNPr-D1 increases the duration of lag phase in Aβ aggregation kinetic. These results show that GNPr-D1 could promote an inhibitory effect on the Aβ aggregation kinetics.

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98) Cholesterol effect on infection mechanism of Infectious Salmon Anaemia Virus.

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Infectious salmon anaemia virus (ISAV) is a pathogen known to cause major problems in the aquaculture industry in Chile, mainly affects the Atlantic salmon (*Salmo salar*). ISAV infection process is performed through the fusion of the viral membrane with the plasma membrane of the target cell. This process is mediated by the ISAV fusion protein located in the viral lipid-bilayer envelope. The role of the structure and physical properties of the host cell membrane on the virus fusion mechanism is still unclear. In this study the effect of membrane host cells cholesterol content on the fusion mechanism was analyzed.

The SHK-1 cell line was used for the assay of fusion and infection. The cholesterol in membranes was extracted by different concentrations of methyl-β-cyclodextrin (MβCD) prior to each assay. Cytotoxicity was measured by MTS assay. Cholesterol content was measured used CHOD-PAP method. This quantification was analyzed on treated and untreated SHK-1 cell. The viral fusion was evaluated by fluorescence-dequenching assays used octadecyl rhodamine B. Quantitative RT-PCR was used to determine mRNA expression of ISAV post-infection with cells and virus treated.

The results de cytotoxicity showed that cell viability did not differ significantly between treated and untreated cell. We found that the cholesterol content of the vesicles affects the fusion of the membranes mediated by ISAV and previous studies shown that the infection is reduced when ISAV treated with MβCD.

Our results demonstrate that the infection and mechanism of fusion ISAV have a dependence on the cholesterol content of the membranes.

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100) Dual-specificity Tyrosine Phosphorylation-regulated Kinase 1A (Dyrk1A) phosphorylates and inactivates Glycogen Synthase Muscular Isoform (MGS) in HeLa cells.

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Glycogen is a multibranched polysaccharide of glucose that serves as a form of energy storage in various cell types. Among the mechanisms that regulate its synthesis are the changes of the phosphorylation state of Glycogen Synthase (GS). Muscular isoform of GS (MGS) is inactivated by phosphorylation, where site 3a is the most important (Ser640) and susceptible to be phosphorylated by different kinases. Glycogen levels in different tumour and cancer cells are negatively correlated with proliferation rates, suggesting that glycogen is consumed for sustaining cancer's growth and survival. DYRK family of kinases are proteins that phosphorylate their substrates in serine and threonine residues. There is in vitro evidence which suggests that DYRK1A, DYRK1B and DYRK2 phosphorylate site 3a of MGS, which could represent a novel regulation mechanism of glycogen synthesis. To determine whether DYRK1A/1B play a role in the regulation of glycogen metabolism in cancer cells, HeLa cell line was used as a study model. Using confocal microscopy, partial co-localization of DYRK1A with MGS was observed in cytoplasm. The interactions of DYRK1A and DYRK1B were further confirmed with co-inmunoprecipitación assays. The physiological relevance was assessed using INDY or harmine, specific inhibitors of DYRK1A/1B to evaluate both the phosphorylation state of MGS in site 3a using Western blot analyses and glycogen levels using an amyloglucosidase-based assay. Together, these results suggest that the inactivation of MGS by DYRK1A is an alternative pathway for MGS regulation in these cells.

102) Blockage of A3 Adenosine Receptor decrease the expression of cell migration/invasion-related genes in Glioblastoma Stem-like Cells under Hypoxia.

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Introduction: Glioblastoma Multiforme (GBM) is a highly invasive neoplasm comprised of a cell subpopulation with a tumorigenic capacity called Glioblastoma Stem-like Cells (GSCs). The hypoxic niche within tumors promotes the Epithelial-Mesenchymal Transition (EMT) and cell migration/invasion through the activation of the A_3 Adenosine Receptor (A_3AR); however, the signaling pathway involved in this processes in GBM remain unknown. The aim of this study was to determine cell migration/invasion-related genes and signaling pathways implicated on the cell migration/invasion regulated by A3AR in GSCs under hypoxia.

Methodology: GSCs of the U87MG human GBM cell line were cultured under normoxia (21% O_2) and hypoxia (0.5% O_2) by 24 hrs. The expression of HIFs (HIF-1α/HIF-2α), EMT markers and cell migration/invasion-related signaling pathways were evaluated by RNAseq and western blot. The contribution of A_3AR on the expression of EMT markers and cell migration/invasion-related genes was evaluated using the A_3AR antagonist, MRS1220.

Results: RNAseq analysis showed that MRS1220 decreased the expression of 15 transcripts directly related with migration/invasion process under hypoxia. A signaling pathway analysis shows that EMT transcriptional factors (TWIST/SNAIL), PI3K/Akt and MEK/Erk pathways may be regulated by the A_3AR.Protein levels of HIFs, EMT markers, p-Erk, and p-Akt increased under hypoxia, and the use of MRS1220 decrease this increment.

Conclusions: We conclude that A_3AR activates PI3K/Akt and MEK/Erk signaling pathways which could regulate HIFs, Twist/Snail levels and, subsequently enhance the EMT, increasing cell migration/invasion-related genes expression in GSCs under hypoxia.

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104) Differential expresion of megalin receptor in breast cancer tumors according to the tumoral subtype.

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Clinical and epidemiological studies in breast cancer have associated increased serum concentrations of 25-hydroxy-vitamin D, with a better prognosis of patients. Also, an increased risk of breast cancer in patients with decreased Vitamin D serum concentrations has been described. Even though the apparent relevance of Vitamin D in breast cancer, no reports have been published in relation to the mechanism of Vitamin Dinternalization and its metabolism in this tissue. It is well known that in the kidney, internalization of the circulating Vitamin D is accomplished by megalin receptors in the cell membrane. Our aim was to analyze the presence of megalin receptor in breast cancer tumor tissues and to evaluate any differences in its expression in the different tumor subtypes. For this purpose we used immunohistochemistry to analyze the expression and localization of megalin in both normal and breast cancer tissue. Further, we evaluated the expression of megalin in T47D (luminal), HCC1937 (triple negative) breast cancer cell lines by immunofluorescence. This study showed that megalin is localized apically in epithelial cells in normal breast, as described previously in kidney. In the luminal breast cancer subtype, megalin was found mainly in the cellular membrane whereas in triple negative tumors there was a greater cytoplasmic localization. In cell lines, both T47D and HCC1937 express megalin endogenously. In these cells, megalin is localized in the cytoplasm, suggesting receptor activity. Our results showed that the megalin expression in breast tumors changes according tumor subtype. CONICYT21161571.

106) Expression analysis of parvovirus derived endogenous viral element in Cavia porcellus.

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Viral infection of the germ line can lead to viral genes or genomes becoming integrated into chromosomes and inherited as host alleles known as endogenous viral elements (EVEs). Most EVEs in animal genomes are sequences originated from retroviruses but, over recent years it has become clear that sequences derived from all seven Baltimore classes occur in animal genomes. Some EVEs present in animal genomes have maintained intact open reading frames (ORFs), and the proteins they encode have been co-opted to perform cellular functions. Parvoviral derived EVEs with intact ORFs have been found in the genome of several animals. We have demonstrated that a parvoviral EVE with an intact ORF is transcribed in the liver of *Octodon degu*. Further analysis of available genomes lead us to find a parvoviral EVE with an intact ORF in the genome of *Cavia porcellus*. We verify that the predicted EVE is present in the genome of *C. porcellus* by PCR amplification of the EVE and its genomic context and subsequent sequencing of the amplicons.

Interestingly, the protein encoded by the EVE is predicted to be express as a fusion with the C-terminal portion of Myosin9 heavy chain. We have detected the presence of a transcript corresponding to the EVE in several tissuesof *C. porcellus*. We are currently trying to determine if the fusion transcript exist, if this is true it will be a strong suggestion of co-option of the parvoviral EVE in *C. porcellus.*

108) MELATONIN MODULATES THE CLOCK GENE EXPRESSION BMAL1, PER1-2 AND WEE-1 IN CULTURE OF HUMAN PLACENTA.

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(Sponsored by CONICYT-79112027 (Chile), Beca Investigación Postgrado UBB-2015 And Colegio Concepción de Chillán.)

Introduction: The coordinated function of the circadian system and the cell cycle is critical for cell development and homeostasis via clock genes Clock, Bmal1, Per1-3 and Cry1-2 and of output genes like cell cycle gene Wee1. Clock genes are expressed ex vivo in placenta, but it is unknown whether the placenta showed crosstalk between circadian system and cell cycle gene Wee1. Methodology: Placentas (38 mg/explants) were obtained at 08:00hrs and maintained in medium alone (M-199) and with melatonin 10nM. Three explants were obtained every four hours and stored in TRIzol Reagent (Invitrogen). RNA expression was measured from cDNA by quantitative PCR Real-Time using the DDCt method and Cyclophilin gene.

Results: The Bmal1, Per1-2, Wee1 maintained their expression in culture for at least 36 hours. Bmal1 showed a peak early in the morning. and melatonin induces the expression of Bmal1 during the night at 03-hrs (P Per2 expression showed a peak at 19 hours the first day and melatonin increases the expression at 19 and 03hrs (P Bmal1/Wee-1 ratio suggesting opposite phases during the culture, while the presence of melatonin, inhibit the Bmal1/Wee-1 ratio($P < 0.05$, ANOVA).

Conclusion: We detected the expression of clock genes of Bmal1, Per1-2, and the cell cycle gene Wee1 in the culture of the placenta. Melatonin inhibits the expression of clock genes and Wee1, suggesting to placenta as a peripheral circadian oscillator synchronized by melatonin.

110) Capillary electrophoresis for determination of nucleotides

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Many cell types release nucleotides into the extracellular environment, which can interact with specific cell-surface receptors and affect many biological processes as placental villi. Nucleotides are rapidly metabolized locally by ecto-nucleotidases with a role in regulation of purinoceptor-mediated response. These enzymes include ecto-ATP-diphosphohydrolase and ecto-5´-nucleotidase. While ADP is a potent activator of platelet aggregation, ATP and adenosine inhibit ADP-induced platelet aggregation.

Human placental cotyledons of human placentas were perfusate with calcium nucleotides injected in the arterial cannula, and collected from a venus cannula. Hydrolyzed nucleotides products were meaured by Capillary Electrophoresis (CE) ATP, ADP, AMP meaurements were performed using uncoated fused-silica capillaries at 20-22 kV in 0.1 M phosphate buffer pH 7.5. The detection was done at 254 nm. Capillary electrophoresis analysis was performed in a Ion Analyzer Capillary Electrophoresis system. Our results confirmed the presence of ecto-ATP-diphosphohydrolase activity in the placental vascular system.

On the other hand, we utilized CE for the determination of glycogen synthase activity in frog oocytes to replace both spectrophometric and radioactive methods avoiding the use of auxiliary enzymes or radioactive substrate. The enzyme catalyzes the incorporation of UDP-glucose into glycogen. The conditions for UDP and UDP-glucose determination included 20 mM tetraborate buffer, pH 9.2 using also detection at 254 nm. The incorporation of UDP-glucose into glycogen followed by CE showed to be a novel approach to measure glycogen synthase activity allowing to measure kinetic parameters and compare the resulats with traditional methods, validating the use of this new methodology, less contaminant and sensitive enough.

112) Neuroendocrine effects of endocrine disruptors on gene expression in Cyprinus carpio.

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The effects of environmental changes on the neuroendocrine system are not well known. Here the effect of three types of endocrine disruptors, heavy metal, estrogen and polycyclic aromatic hydrocarbon was determined by RT-qPCR analyses of a panel of hypophyseal factors in *Cyprinus carpio*, a model organism for environmental monitoring. In three different experiments adult male carp were injected on three consecutive days intraperitoneally with 0.5 mg/kg $ZnCl_2$, 0.5 mg/kg 17b-estradiol, 0.5 mg/kg benzo-A-piren (BaP), a control group with respective vehicle, and processed on the fourth day. All analyses were performed in duplicate and normalized for *ef*1a expression. Modulation of gene expression of hormones prolactin (PRL), growth hormone (GH), somatolactin (SL) a and b, transcription factors Pit-1, MTF-1 and homeostatic genes metallothionein (MT), cyp1A revealed effects involving MTF-1, estrogen receptor and arylhydrogen receptor pathways acting via respective metal (MRE), estrogen (ERE) and xenobiotic (XRE) response elements. Interestingly, SLa and b showed differential expression when treated with estrogen and BaP, thereby highlighting complex organismal response. Taken together the results showed that selected marker gene expression could serve as early indicator of endocrine disrupting effects to assess biological relevant changes in the aquatic environment

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114) In silico analysis of two alpha expansin proteins involved in cell wall disassembly during ripening of two related fruit species.

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Expansins are proteins associated to several biological processes in plants including fruit ripening. Changes in the cellulose-hemicellulose fraction take place during ripening of *Fragaria chiloensis* and *Fragaria ananassa* fruits, two species of Fragaria genus with different softening rates. Previously was reported the increase of some expansins transcripts during fruit softening with FaEXPA1 showing a greater increase in accumulation respect to FcEXPA1. In order to understand at the molecular level the differences in expansin mechanism of these two proteins, we performed a structural characterization of two softening related α-expansins. FcEXPA1 and FaEXPA1 proteins showed similar 3D structures, and displayed the two characteristic domains; cellulose-binding domain with a β-sandwich structure and the catalytic domain with a β-barrel structure. However, the open groove (in the center of the protein) showed differences in size and shape. Additionally, the conformational interaction between the proteins and cellulose as ligand was carried out by molecular dynamics simulation (MDS). FaEXPA1 open groove is larger, in agreement with its higher selectivity for cellulose as substrates evaluated by MDS. Different orientation of cellulose regarding to the open groove of each protein was observed, because in FcEXPA1 the ligand display a break in the linear structure. The MDS showed a favorable free binding energy in the two protein-ligand complexes evaluated; being FaEXPA1-cellulose the complex of greater affinity. Finally, the two complexes showed that van der Waals forces as major contribution to the protein-ligand interaction.

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116) Development of photolyase activity method based on ion pair reagent coupled to RP-UHPLC-MS/MS.

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Chronic exposure to sunlight generate DNA damage causing skin cancer or photo-aging mainly due to formation of cyclobutane pyrimidine dimers (CPDs), altering skin cell viability. Nevertheless, CPDs are reverted by endogenous mechanism, but they can induce mutations. Exogenous enzymes like photolyase reverts CPDs. Photolyase is a flavoprotein that use blue-light photons as an energy source to cleave CPDs, restoring DNA and avoiding mutations. Therefore, photolyase is being incorporated to film-forming medical devices and cream formulations. For this reason, the measurement of photolyase activity is a crucial step, however, the methods are time consuming. We develop a fast, selective and sensitive photolyase activity assay based on determination of the reaction product (15pb oligonucleotide) by Ion Pairing (IP)-RP-UHPLC-ESI-MS/MS(MRM). The substrate is a 15bp oligonucleotide containing a central CPD. Three IP reagents (triethylamine, N-ethyldiisopropylamine and hexylamine), in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP)-methanol buffer, were evaluate to obtain higher MRM transition intensity, optimizing their concentration by Response Surface Methodology (RMS). Chromatography separation of substrate/product was performed varying the methanol gradient. The method was validated with a cyanobacteria photolyase enzyme. Optimized IP reagent and HFIP showed on Q1 scan that trimethylamine and N-ethyldiisopropylamine favours the parent ion oligonucleotide with more negative charges, otherwise hexylamine decreases it. Based on this results, we described the selection of MRM transition, optimization of chromatographic conditions and analytical parameters for each IP/HFIP in the quantification of 15bp oligonucleotide. This method enable identification of new sources of photolyase and the quality control of products that prevent skin cancer and photo-anging.

118) Steady-State kinetic study of substrate inhibition and product activation in the ADP-dependent phosphofructokinase/glucokinase from Methanococcus maripaludis.

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In some archaeal glycolysis, glucokinase and phosphofructokinase activities utilize ADP instead of ATP as phosphoryl donor. In organisms belonging to *Methanococcales* one enzyme performs both activities. Until now, all these enzymes have been reported as nonregulated. Nevertheless, recently we found that the bifunctional PFK/GK enzyme from *Methanococcus maripaludis* (MmPFK/GK) is activated by AMP (reaction product) and also presents substrate inhibition by glucose.

In this work, GK activity was measured at different [glucose] at several fixed MgADP and AMP concentrations. Analysis of these experiments shows a substrate inhibition effect when glucose increase above 100 mM, and this inhibition effect is enhanced with an increment of [AMP].

In order to obtain V_{max}, K_M and K_i values for each fixed [MgADP] and [AMP], a canonical substrate inhibition model was used. Based on the analysis of V_m and K_m as a function of [MgADP] at fixed [AMP], an ordered sequential mechanism was assigned, with MgADP as the first substrate to bond. Additionally, substrate inhibition can be explained by formation of a dead-end complex between free enzyme and glucose.

At fixed [MgADP], an increase of [AMP] causes a hyperbolic decrease of both K_m and K_i for glucose. Taking this into account, we conclude that activation by AMP of MmPFK/GK occurs mainly through a modification of glucose affinity. Also, the increment of substrate inhibition produced by AMP can be explained as an increase in glucose affinity of the free enzyme, driving the formation of the nonproductive enzyme-glucose complex. Fondecyt 1150460.

FONDECYT 1150460.

120) Role of DYRK1A and DYRK1B in the regulation of Muscle Glycogen Synthase in cell components of seminiferous epithelium.

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(Sponsored by FONDECYT 1141033 (JCS), DID UACh, Beca CONICYT KV, MECESUP AUS 1203 KV)

Dual-specificity Tyrosine Regulated Kinases (DYRKs) family contain five members (1A, 1B, 2, 3, 4) that possess Ser/Thr kinase activity. They have sequence and functional similarities, are distributed in many tissues and present different substrate specificities. DYRKs are highly expressed in testis but little is known about their roles. The purpose of this work was to analyze the implication of DYRK family on the regulation of Muscle Glycogen Synthase (MGS), enzyme responsible of glycogen synthesis in cellular components of seminiferous epithelium. First, we detected differential expression of five DYRKs by qRT-PCR observing that DYRK1A is highly expressed in adult Sertoli cell line, spermatogonia and spermatocytes (GC-1, GC-2). Meanwhile at protein level, DYRK1A and DYRK1B are the most abundant according to Western blot analysis. Through immunofluorescence and subcellular fractionation, we evaluated the distribution of DYRK1A, DYRK1B, MGS and inactive MGS phosphorylated on Ser641. These proteins share the same location in the cell and could also interact. This was confirmed by immunoprecipitation assays. Pharmacological inhibition of DYRK1A and DYRK1B with Indy (Inhibitor of DYRK) for24 hours, showed a reduction of the phosphorylated form of MGS and this decrease was even greater in co-treatment experiments with BIO, an inhibitor of GSK3B. With these results, we can suggest that DYRK1A and DYRK1B have a regulatory role on glycogen synthesis, phosphorylating Ser641 and triggering the inactivation of the enzyme in these cells.

122) Generation of DNA double strand breaks in hematopoietic cells: Role of β-catenin and Topoisomerase IIα.

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Wnt/β-catenin signaling plays a crucial role in hematopoiesis and deregulation of its activity is key in the development of leukemias, where genomic instability is a hallmark. Elevated transcriptional rates are involved in genomic instability by increasing DNA breaks. Here we examine Wnt/β-catenin signaling on DNA break generation and its possible interaction with topoisomerases II (topo IIα and IIβ), as these enzymes play an essential function in cleavage and re-ligation of double-stranded DNA during replication, transcription and DNA damage repair. KG-1 human hematopoietic cells were treated with CHIR, a Wnt/β-catenin signaling activator, and the interaction of β-catenin with topo IIα and/or IIβ was examined by co-immunoprecipitation (co-IP) and immunofluorescence. Likewise, γ-H2AX, which measure DNA breaks, and its co-localization with β-catenin, topo IIα and topo IIβ was determined by immunofluorescence. Single cell electrophoresis (Comet assays) was also performed to evaluate DNA damage. DNA breaks were readily detected with γ-H2AX and comet assays upon induction of the signaling cascade. Likewise, we observed co-localization of β-catenin with topo IIα and IIβ soon after CHIR treatment. Interestingly, γ-H2AX foci co-localized with β-catenin and topo IIα, but not with topo IIβ. Our results suggest that transcriptional stress induced by Wnt/β-catenin signaling may be responsible for DNA break generation and genomic instability in hematopoietic cells and the subsequent malignant transformation to leukemia.

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124) Evaluation of the importance of putative mitochondrial iron transporters in reproductive development of Arabidopsis thaliana.

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Among the essential micronutrients for plants, iron is required in greater abundance, mainly because it is used in indispensable processes such as respiration and photosynthesis. Different studies have established a clear dependence between iron and plant fertility. At the histological level it has been determined that anthers and pollen accumulate large amounts of iron. On the other hand, different mutants in iron transport have fertility problems. In this context, mitochondrial activity plays an essential role both in the synthesis of iron cofactors and in the development of pollen. Recently, the first plant Mitochondrial Iron Transporter (MIT) has been described in *Oryza sativa*. *OsMIT* is an essential gene, and *Osmit* heterozygous mutant plants exhibit a decrease in mitochondrial iron content and an imbalance of iron homeostasis in whole plants. We have now identified two putative homologous genes in Arabidopsis, *AtMIT1* and *AtMIT2*. Homozygous mutant plants were obtained for both genes, and none of these showed a visible altered phenotype, suggesting they have redundant functions. Double heterozygous mutant plants were obtained and are under analysis. They show a reduction in seed set, with the presence in siliques of an increased number of unfertilized ovules and/or aborted embryos at an early development stage. These results suggest that double mutants (*mit1/mit2*) could not be obtained and that the presence of at least functional *MIT* genes is essential.

126) Expression and purification of glut1 for biophysical characterization.

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GLUT1 is a membrane protein that facilitates the translocation of glucose across the membrane. Because this protein plays a critical role in glucose homeostasis and metabolic diseases, understanding the molecular mechanism of glucose transport is of utmost relevance. The recent publication of GLUT1's crystal structure provides a new framework for understanding the structural details of the transport mechanism, including the interactions of the transporter with its substrate and immediate surroundings. Here, we generated different constructs of GLUT1 for biophysical characterization *in vivo* and *in vitro*. To test overexpression and cellular localization, we monitored the fluorescence signal of a GLUT1-GFP construct using epifluorescence and TIRF microscopy. We tested functionality by measuring kinetic properties of sugar transport in cells overexpressing the transporter. Finally, we overexpressed GLUT1 carrying a His-tag for purification using cellular fractioning followed by affinity chromatography, and set up a purification protocol into mild detergents. While purification of this transporter into mild detergents will allow its reconstitution into other membrane mimetic systems for biophysical studies *in vitro*, GFP-labeled protein can be used in combination with TIRF microscopy to test lateral diffusion and stoichiometry directly in living cells.

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128) Identification of tenascin C (TNC) as a target gene of Wnt/β-catenin signaling in hematopoietic cells.

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Acute myeloid leukemia (AML) is the most common type of leukemia. Since an abnormal activity of Wnt/β-catenin signaling in hematopoietic cells was observed in leukemia-stem-cells, the search for new target genes of the cascade will allow for a better understanding of the onset/progression of the disease. Here we present genome-wide differential expression analyses in KG-1 AML cells induced by CHIR, a Wnt/β-catenin signaling agonist. RNASeq experiments were carried out at 4 and 48h. RNA was processed using the Illumina TruSeq Stranded Total RNA with Ribo-Zero ribosomal RNA depletion kit. RNASeq was performed using the Illumina HiSeq2500 with a depth >120 million pair-end reads (2x50 bp). Reads were aligned with TopHat2, differential gene expression was performed with DESeq2 and protein-protein interaction network identification with STRING (v10.5) and visualized with Cytoscape (v3.5.1). A clear dose and time dependence on β-catenin stabilization was observed in these cells after incubation with CHIR. After short-term treatment in KG1 cells with CHIR for 4h (n=4), we identified 209 differentially expressed genes (128 and 81 genes, up- and down-regulated, respectively; $P<0.05$). Similarly, experiments at 48h (n=3) revealed 146 and 116 genes were up- and down-regulated, respectively. Notably, the cell adhesion molecule tenascin C (TNC, Chr9) was the only gene significantly up-regulated in both conditions (Benjamini and Hochberg correction for multiple testing). Our results suggest that *TNC* is a Wnt/β-catenin target gene and support a role for this gene in tumor growth as is likely observed in the onset/development of leukemia.

FONDECYT Postdoctorado 3150612 to M.F.V. and FONDECYT Regular 1140353 to G.D.V.

130) Profibrotic response of the pathway oxldl/lox-1 in cardiac myofibroblast.

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Introduction: After myocardial infarction, the myofibroblasts (CMF) appear and are perpetuated in the heart. These cells secrete high levels of extracellular matrix proteins, which generates a long-term cardiac fibrosis that culminate in heart failure. The oxidized low-density lipoprotein (oxLDL) is an important cardiovascular risk factor. Its mechanism of action is mediated by LOX-1 receptor in other cells types. In LOX-1 knockout mice subjected to ischemia/reperfusion were observed increase in survival and a decreased in collagen type I. We propose that the oxLDL/LOX-1 pathway increases the profibrotic phenotype of cardiac myofibroblasts.

Methodology: ***Cell culture of MFC***:MCF were obtained from cardiac fibroblast differentiated with TGF-β1 (10 nM) by 96 hours. ***Treatments***:(a) MFC were treated with oxLDL (10 ug/mL) by 24 and 72 hours (control: untreated MFC to each time) (b) MFC were pre-treated with lovastatin (100 nM) by 30 minutes (LOX-1 inhibitor) and subsequently treated witch oxLDL by 24 and 72 hours**.** ***Determination of level and expression fibrotic markers***: were evaluated by western blot and qPCR: COL-1, FN-EDA, alpha-SMA, TGF-β1, TGF-β receptor**.** ***Immunocytochemistry***: morphology (alpha-SMA) and proliferation (ki67) were determinate by confocal microscopy.

Results: MCF treatment with oxLDL maintained the levels of alpha-SMA and increased the levels of COL-1, FN-EDA. The morphology of MFC treated with oxLDL was smaller that cells untreated and these cells were ki67 positive. Pre-treatment with lovastatin prevented effect profibrotic of oxLDL.

Conclusion: Treatment of MFC with oxLDL increases the profibrotic phenotype via LOX-1 receptor.

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132) Evolutionary constraints determine three-dimensional domain swapping in the DNA-binding 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 domain structures of Fox proteins have been described as monomers in the presence of DNA, members of the FoxP subfamily show dimeric structures 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 those observed in the isolated monomer. Experimental evidence from our group suggests that monomeric FoxP1 undergoes local structural rearrangements rather than extensive unfolding, in dramatic contrast to the established consensus about the DS mechanism. In order to understand the evolution of this mechanism in Fox proteins, we performed a computational and biophysical study of FoxP subfamily. Phylogenetic and ancestral reconstruction studies show that FoxP members and their last common ancestor (AncP) have the conserved proline-to-alanine substitution in a hinge loop motif (FPYF) of monomeric Fox proteins, which has been described as required to enable DS. Size exclusion chromatography experiments showed that despite the high sequence identity between FoxP proteins, the dissociation constants (Kd) determined are in a micro to milimolar range. Preliminary unfolding experiments of FoxP proteins show a three-state mechanism in FoxP2 and FoxP4 ($N \leftrightarrow I \leftrightarrow U$), while the obligate dimeric FoxP3 unfolds via a two state mechanism ($N_2 \leftrightarrow 2U$). Molecular dynamics of AncP and FoxP proteins suggest that key substitutions in ancP affect the dynamics which could explain differences in the folding mechanism and oligomerization propensity in FoxP subfamily.

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134) Transcriptome analysis of coding- and noncoding RNAs from the Stomach Adenocarcinoma TCGA study reveals potential ethnogeographic component in tumor-adjacent tissues.

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Background: Gastric cancer is one of the leading causes of cancer-death worldwide. A specific sequence of lesions precedes the onset of gastric cancer. The characterization of differentially expressed transcripts between tumor and tumor-adjacent tissue samples may help shed light on molecular mechanisms driving the progression of the preneoplastic lesions.

Methods: MicroRNAseq data from 41 paired tumor and tumor-adjacent samples from the Stomach Adenocarcinoma TCGA study were downloaded from the Genomic Data Commons using TCGA-Assembler within R, and compared for differential expression analysis using edgeR. Unsupervised clustering by principal component analysis revealed a striking segregation of tumor-adjacent tissues into 3 groups by tissue source site, which was not observed in the tumors. Subsequently, the same phenomenon was observed for differential expression analysis of RNAseq data from 32 paired tumor and tumor-adjacent tissues from the same study. Functional enrichment of microRNA clusters corresponding to each group, using miRpath V.3, suggested that some of the altered signaling pathways involved in the segregation of samples include viral carcinogenesis and epithelial response to bacterial pathogens.

Discussion: Though further research is warranted, these data suggest more variability in the precancerous lesions than tumor samples, possibly due to environmental conditions to which the stomach epithelial cells are exposed in different geographic regions.

136) Effect of IL-4/13A on the phagocytic capacity of salmonid leukocytes.

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The profesional phagocytes described on teleost fish are neutrophils, macrophages, dendritic cells, and B lymphocytes. The role of cytokines stimulating phagocytic capacity has been described in superior vertebrates but little is known about this function in fish. Actually, it has been described that rIL-4/13A increases phagocytosis in Japanese pufferfish and Grass carp, however the function of this cytokine in salmonids has not been studied. In this work we evaluated the effect of rIL-4/13A on the phagocytic capacity and reactive oxigen species (ROS) production in salmonid cell lines and primary culture of salmon spleen B cells. Phagocytosis was assayed with fluorescent latex beads and the analysis was done by flow cytometry. ROS production was tested with the probe 2,7-dichlorohydrofluroescein diacetate and also analyzed by flow cytometry. The results showed that rIL-4/13A increases phagocytosis by SHK-1 and RTS-11 cell lines, which are macrophage-like cells of salmon and trout, respectively. The cytokine also also induced phagocytosis in salmon spleen B lymphocytes. ROS production also enhances in RTS-11 and B cells suggesting that microbicidal activity is also induced in this phagocytes. In conclusion, rIL-4/13A increases the phagocytic capacity and ROS production in salmonid leukocytes, suggesting that this cytokine has a biological role in pathogen clearence in Atlantic salmon and rainbow trout.

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138) Molecular Diagnostic for CagA in Stool Samples Positive for Helicobacter pylori.

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Introduction: *Helicobacter pylori* infection is extremely common in Chilean patients (70%) and is a factor for gastritis and peptic ulcers, as well as gastric cancer. Our laboratory has developed a non-invasive method for testing for the infection using nested-qPCR on stool samples for the *H. pylori* UreC gene. We aim to develop a similar non-invasive method of diagnosis for the cytotoxin-associated gene A antigen (CagA) gene, which is associated with more virulent strains of the bacteria and higher probability of gastric cancer.

Methods: DNA was extracted from fecal samples of patients with gastric associated pathologies, and *H. pylori* infection, confirmed both by nested-qPCR for UreC and histology-Giemsa. A semi-nested-qPCR (conventional PCR + qPCR) was used to detect the presence of CagA, and therefore more potent strains of the bacteria. The strain 26995 of *H. pylori*, positive for CagA, served as a positive control.

Results: The semi-nested-qPCR method successfully amplifies the CagA gene from *H. pylori*, and thus we can detect the incidence of virulent strains in infected patients. Of the 97 UreC+ samples tested, only 16 were also CagA+ (16,5%).

Conclusion: The method of diagnosis of CagA in stool samples proves to be a potent tool for detecting virulent strains of the bacteria *H. pylori*. It appears that this population has a lower rate of CagA positive strains, as compared to areas with higher rates of gastric cancer. We look forward to exploring reasons why prevalence of CagA positive strains seems to be decreasing in our population.

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140) Transcriptomic modulation of long non-coding RNAs associated with stress response and growth in the skeletal muscle of the fine flounder (Paralichthys adspersus).

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Previous studies characterized the transcriptomic response associated to confinement-stress stimulus in the skeletal muscle of the Chilean flounder *Paralichthys adspersus*. However, until now there was no information available on the involvement of long non-coding RNAs(lncRNAs) in chronic-stress response and muscle growth. Analysis of the sequenced data between stressed and unstressed samples taken at 4 and 7 weeks (4w and 7w) were performed using CLC Genomics Workbench software. A custom pipeline was developed to identify lncRNAs within a previously obtained transcriptome from *P. adspersus* muscle tissue. Contigs with an average coverage less than 50 reads, with ORF above 200 nt and with significant coding potential were removed from further analysis. In addition, contigs matching with protein and nucleotide databases, as well as those identified using the NCBI batch web CD-search tool were also removed. The transcripts remaining after this filtering pipeline were considered putative lncRNAs. After the custom pipeline, a total of 1,031 putative lncRNAs were identified. These discovered *P. adspersus* non-coding transcriptspresented the typical features of lncRNAs such as lower values of GC content, length and transcriptional activity, compared with the coding transcripts. The transcriptional responses of lncRNA (stress vs control) revealed an important downregulation at 7w compared with 4w. Furthermore, the validation results of selected differentially expressed ($|FC^{3}4|$, $p<0.01$) correlated lncRNAs/mRNAs pairs showed good adjustement values between RNA-seq and RT-qPCR values ($r^2=0.72$ and 0.74 for lncRNAs and mRNAs, respectively; $p<0.01$). Overall, our findings provide the first characterization of lncRNAs in fish skeletal muscle and stress.

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