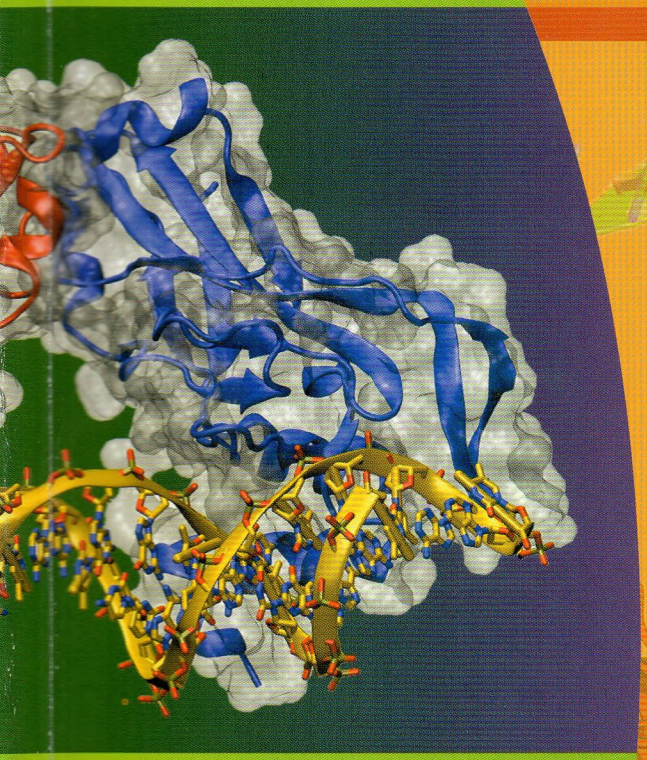
SOCIEDAD DE BIOQUÍMICA Y BIOLOGÍA MOLECULAR DE CHILE



XXXV REUNIÓN ANUAL

Centro de Convenciones Dreams, Puerto Varas • 2 - 5 de octubre 2012

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PROGRAM

Tuesday, October 2nd

10:30- 13:00 Registration
13:00- 14:30 Lunch

15:00 -17:00 **ORAL SESSION 1**

STRUCTURE AND FUNCTION OF MACROMOLECULES
Salón Puerto Varas

Chair: Francisco Melo
Co-chair: Mónica Salas

15:00 **RNA aptamers capable of inhibiting isoform LI2 of spider venom sphingomyelinase D (SMD-LI2). Salinas-Luypaert, C.**[1], Sapag, A.[1]. Laboratory of Gene Pharmacotherapy, Department of Pharmacological and Toxicological Chemistry, Faculty of Chemical and Pharmaceutical Sciences, Universidad de Chile, Santiago, Chile[1].

15:15 **A mechanism for the substrate inhibition of pig kidney fructose-1,6-bisphosphatase: mixed-subunit oligomers and tryptophan-containing mutants allow two different classes of binding sites to be distinguished. Asenjo, J.L.**[1], Ludwig, H.C.[1], Droppelmann, C.A.[1], Cárcamo, J.G.[1], Cárdenas, M.[2], Concha, I.I.[1], Yáñez, A.E.[1], Cornish-Bowden, A.[2], Slebe, J.C.[1]. Instituto de Bioquímica y Microbiología, Universidad Austral de Chile.[1], Bioénergétique et Ingénierie des Protéines, Institut de Microbiologie de la Méditerranée, CNRS, Aix-Marseille Université, Marseilles, France[2].

15:30 **Atomic model of the core of *Gracilaria chilensis* phycobilisome. Dagnino-Leone, J.**[1], Figueroa, M.[2], Bunster, M.[1], Martínez-Oyanedel, J.[1]. Laboratorio de Biofísica Molecular, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción[1], GIGA-Research, Molecular Biology and Genetic Engineering Unit, University of Liege. Belgium[2].

15:45 **Towards deciphering the protein-DNA recognition code by statistical analysis of amino acid-base contact propensities. Schüller, A.**[1,2], Melo, F.[1,2]. Molecular Bioinformatics Laboratory, Millennium Institute on Immunology and Immunotherapy[1], Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile[2].

16:00 **Structure-based prediction of transcription factor binding specificities using a Metropolis-Montecarlo simulation approach. Norambuena, T.**[1,2], Melo, F.[1,2]. Molecular Bioinformatics Laboratory, Millennium Institute on Immunology and Immunotherapy[1], Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile[2].

16:15 **RNABPviewer, a software tool for the three-dimensional analysis and visualization of canonical and non-canonical base pairs in RNA structures. Cares Galvez, J.**[1,2], Rodríguez, F.[1,2], Melo, F.[1,2]. Molecular Bioinformatics Laboratory, Millennium Institute on Immunology and Immunotherapy, [1], Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile[2].

16:30 **Comparative modeling of B-DNA structures. Ibarra, I.**[1,2], Madhusudhan, M.[3], Melo, F.[1,2]. Molecular Bioinformatics Laboratory, Millennium Institute on Immunology and Immunotherapy[1], Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile[2], Biomolecular Modeling and Design Division, Bioinformatics Institute, 30 Biopolis Street, Singapore[3].

16:45 **Energy and structural characterization of the interaction between Smac/DIABLO N-terminal peptides and human Survivin using bioinformatics tools. Acevedo, W.**[1], Guzmán, L.[1], Aguilar, L.F.[1]. Instituto de Química, Pontificia Universidad Católica de Valparaíso. Avenida Universidad #330. Campus Curauma, Valparaíso[1] Sponsored by Guzmán, L.

15:00 -17:00 **ORAL SESSION 2**

MOLECULAR BIOLOGY OF THE CELL I
Salón Frutillar

Chair: Raúl Herrera
Co-chair: Claudio Acuña

15:00 **Establishment of primary cultures of advanced cervical cancer as a model for antisense therapy. Avila, R.**[1], Socias, M.[2], Dadlani, K.[3], Zapata, L.[4], Villota, C.[5], Socias, T.[6], Oliveira-Cruz, L.[6], Burzio, V.[7], Bustamante, E.[8], Burzio, L.[5], Villegas, J.[5]. Fundación Ciencia para la Vida, Andes Biotecnologies S.A., Fac. Ciencia Biológicas, Universidad Andrés Bello[1], Clínica Alemana[2], Fundación Ciencia para la Vida[3], Anatomía Patológica, Hospital Barros Luco Trudeau[4], Fundación Ciencia para la Vida, Andes Biotechnologies S.A., Fac. Ciencias Biológicas, Universidad Andrés Bello[5], Fundación Ciencia para la Vida, Andes Biotechnologies S.A.[6], Fundación Ciencia para la Vida, Andes Biotecnologies S.A., Fac. Ciencias Biológicas, Universidad Andrés Bello[7], Fundación Arturo López Pérez[8].

15:15 **Unveiling molecular constituents of a multiprotein complex involved in excitation-transcription coupling in skeletal muscle. Buvinic, S.**[1,2], Almarza, G.[2], Arias, M.[2], Jaimovich, E.[2]. Departamento de Ciencias Básicas y Comunitarias, Facultad de Odontología, Universidad de Chile, Santiago, Chile.[1], Centro de Estudios Moleculares de la Célula, ICBM, Facultad de Medicina, Universidad de Chile, Santiago, Chile[2].

15:30 **A bidirectional and complex relationship between mitochondrial dynamics and insulin signaling in skeletal muscle cells. del Campo, A.**[1], Parra, V.[1], Gutiérrez, T.[1], Morales, P.E.[1], Lavandero, S.[1,2,3]. Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/Faculty of Medicine[1], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile. [2], Department of Internal Medicine, University of Texas Southwestern Medical Center, USA[3].

15:45 **Herp cytoprotective effect against oxidative stress through regulation of the inositol trisphosphate receptor (IP3R). Paredes, F.**[1], Gatica, D.[1], Quiroga, C.[1], Parra, V.[1], Bravo , R.[1], Contreras , A.[1,2], Troncoso, R.[1], Jaimovich, E.[1,2], Lavandero, S.[1,3]. Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/Faculty of Medicine[1], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile[2], Department of Internal Medicine, University of Texas Southwestern Medical Center, USA[3].

16:00 **Effect of testosterone and insuline on unfolded protein response (UPR) and glucose uptake in cultured human endometrial cells. Rosas, C.**[1], Poblete, C.[1], Romero, C.[2,3], Lavandero, S.[4], Vega, M.[2,3]. Endocrinology and Reproductive Biology Laboratory, University of Chile Clinical Hospital[1], Endocrinology and Reproductive Biology Laboratory, University of Chile Clinical Hospital.[2], Obstetric/Ginecology Department, University of Chile Clinical Hospital.[3], Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/ Faculty of Medicine, University of Chile[4].

16:15 **Functional evidence reveals two clathrin light chains in *Arabidopsis thaliana*. Vásquez, B.**[1], Urrutia, P.[1], Norambuena, L.[1]. Laboratorio de Biología Molecular Vegetal, Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile[1].

16:30 **Methylation status of tumor suppressor genes in total DNA sputum samples: a promising tool for early detection of COPD and lung cancer in smokers. Guzmán, L.**[1], Depix, M.S.[2], Salinas, A.M.[2], Roldán , R.[3], Aguayo, F.[4], Alejandra, S.[2], Vinet, R.[5,6]. Instituto de Química, Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile[1], Facultad de Salud, Escuela de Tecnología Médica, Universidad Santo Tomás, Santiago, Chile[2], Unidad de Enfermedades Respiratorias, Hospital San José, Santiago, Chile[3], Programa de Virología, Instituto de Ciencias Biomédicas (ICBM), Facultad de Medicina, Universidad de Chile, Santiago, Chile.[4], Facultad de Farmacia, Universidad de Valparaíso, Valparaíso, Chile.[5], Centro Regional de Estudios en Alimentos Saludables (CREAS), Valparaíso, Chile[6].

16:45 **Dexamethasone stimulates autophagy and metabolic changes in skeletal muscle cells. Troncoso, R.**[1], Paredes, F.[1], Gatica, D.[1], Vasquez-Trincado, C.[1], Rodríguez, A.E.[1], Lavandero, S.[1,2,3]. Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/ Faculty of Medicine, University of Chile[1], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile[2], Department of Internal Medicine, University of Texas Southwestern Medical Center, USA[3].

17:00 - 17:30 Coffe break

17:30 - 19:30 SYMPOSIUM: REGULATORY NETWORKS IN PLANT METABOLISM
Salón Gran Ballroom

Chair: Rodrigo Gutiérrez

17:30 **Systems approaches to unravel plant gene regulatory networks. Grotewold, E.**[1]. Center for Applied Plant Sciences, The Ohio State University, Columbus, OH 43210[1].

18:00 **Mapping plant hormone signaling networks using halo-tag protein arrays. Yazaki, J.**[1,2], Galli, M.[2], Kim, A.Y.[2], Nito, K.[1], Guillen, F.A.[3], Carvunis, A.[4,5], Chang, K.N.[1], Quan, R.[2], Nguyen, H.[2], Song, L.[1], Alvarez, J.M.[6], Gutiérrez , R.A.[6], Schroeder, J.I.[3], Chory, J.[1,7], Ramachandran, N.[8], Braun, P.[9], LaBaer, J.[10], Vidal, M.[4,5], Ecker, J.R.[1,2,7]. Plant Biology Laboratory, The Salk Institute for Biological Studies, 10010 North Torrey Pines Road La Jolla, CA 92037, USA. [1], Genomic Analysis Laboratory, Salk Institute for Biological Studies, La Jolla, CA 92037, USA[2], Division of Biological Sciences, Cell and Developmental Biology Section, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0116, USA[3], Center for Cancer Systems Biology and Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, MA 02215, USA,[4], Department of Genetics, Harvard Medical School, Boston, MA 02115, USA[5], FONDAP Center for Genome Regulation, Millennium Nucleus Center for Plant Functional Genomics, Departamento de Genética Molecular y Microbiología, Pontificia Universidad Católica de

Chile, Alameda 340, Santiago, Chile[6], Howard Hughes Medical Institute, The Salk Institute for Biological Studies, 10010 North Torrey Pines Road La Jolla, CA 92037.[7], Life Technologies Corporation, Carlsbad, CA 92008, USA.[8], Center of Life and Food Sciences, Weihenstephan der Technischen Universitaet Muenchen, Alte Akademie 8 85354 Freising, Germany.[9], The Biodesign Institute, Personalized Diagnostics, Arizona State University, Tempe, AZ 85287, USA[10].

18:30 **Applications of metabolomics in plant systems biology. Kusano, M.**[1,2] RIKEN Plant Science Center[1], Kihara Institute for Biological Research, Yokohama City University[2]

19:00 **Nitrogen regulatory networks controlling plant root growth. Gutiérrez, R.**[1]. FONDAP Center for Genome Regulation, Millennium Nucleus Center for Plant Functional Genomics, Departamento de Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile, Alameda 340, Santiago, Chile[1].

19:30 - 20:45 **OPENING PLENARY LECTURE**

Salón Gran Ballroom

Chair: Roxana Pincheira

19:30 **Modelling new strategies to treat cancer. Evan, G.I.**[1]. Dept. Biochemistry, Sanger building, 80 Tennis Court Rd, Cambridge CB2 1GA, UK[1].

20:45 Cocktail

21:30 Dinner

Wednesday, October 3rd

09:00 -11:00 **ORAL SESSION 3**

PROTEINS STRUCTURE AND FUCTION

Salón Puerto Varas

Chair: Juan Carlos Slebe
Co-chair: Marta Bunster

09:00 **Salsolinol and isosalsolinol: products of dopamine and acetaldehyde condensation as final effectors of the reinforcing effect of ethanol. Berríos-Cárcamo, P.A.**[1], Rivera-Meza, M.[2,3], Buscaglia, M.[2], Zapata-Torres, G.[4], Herrera-Marschitz, M.[3], Israel, Y.[1]. Laboratorio de Farmacoterapia Génica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile[1], Laboratorio de Farmacogenética del Alcoholismo, Facultad de Medicina, Universidad de Chile[2], Biomedical Neuroscience Institute (BNI), Programme of Molecular & Clinical Pharmacology, Institute of Biomedical Sciences (ICBM), Faculty of Medicine, University of Chile[3], Unidad de Gráfica Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile[4]. Sponsored by Sapag, A.

09:15 **Caveolin-1 reduces HIF1a dependent transcription and target gene expression in cancer cells via a mechanism involving nitric oxide. Sanhueza, C.**[1], Silva , P.[1], Diaz, M.I.[1], Avalos, Y.[1], Leyton, L.[1], Quest, A.[1]. Laboratorio de Comunicaciones Celulares, Centro de Estudios Moleculares de la Célula, Facultad de Medicina, Universidad de Chile[1].

09:30 **Role of the electrostatic potential in the cooperativity between pHo-sensors in K2P channel TASK-3. González, W.**[1], Arévalo, B.[1], Zúñiga, L.[2], Niemeyer, M.I.[2], Cid, P.[2], Sepúlveda, F.[2]. Centro de Bioinformática y Simulación Molecular (CBSM), Universidad de Talca[1], Centro de Estudios Científicos (CECs)[2].

09:45 **Kinetic effects of mutations in residues involved in metal binding to the LIM-domain of a rat brain agmatinase-like protein. Benítez, J.R.**[1], Vallejos, A.[1], Montes, P.[1], Cofré, J.[1], Romero, N.[1], Hidalgo, Á.[1], García, D.[1], Martínez, J.[2], Carvajal, N.[1], Uribe, E.A.[1]. Laboratorio de Enzimología, Departamento de Bioquímica y Biología Molecular, Facultad de Cs. Biológicas, Universidad de Concepción.[1], Laboratorio de Biofisica Molecular, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción[2].

10:00 **Comparison and classification of DNA polymerase chains, domains and sub-domains based on multiple sources of structural and sequence information. Slater, A.W.**[1,2], Cifuentes, J.J.[1,2], Melo, F.[1,2]. Molecular Bioinformatics Laboratory, Millennium Institute on Immunology and Immunotherapy[1], Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile[2].

10:15 **Understanding non-trivial protein topologies using simplified molecular dynamics. Ramírez-Sarmiento, C.**[1], Villalobos, P.[1], Baez, M.[2], Guixé, V.[1], Babul, J.[1]. Departamento de Biología, Facultad de Ciencias, Universidad de Chile.[1], Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile[2].

10:30 **LjLTP10 gene coding for Lipid Transfer Protein is involved in aerial organ morphology in *Lotus japonicus* and have a role in cuticle formation and acclimation during drought stress. Tapia, G.**[1], Alcorta, M.[1], Parra, C.[2], Morales, L.[2], Berbel, A.[3], Madueño, F.[3]. INIA-Quilamapu, Chillan, Chile[1], Universidad de Talca, Talca, Chile[2], IBMCP, Valencia, España[3]. Sponsored by Ruiz, S.

10:45 **Knotted and untied topologies of single-chain ARC repressor characterized by optical trap force spectroscopy. Bustamante, A.**[1], Reyes, J.[1], Obando, P.[2], Guerra, D.[2], Wilson, C.A.[3,4], Bustamante, C.[3,4], Baez, M.[1]. Laboratorio de Bioquímica, Departamento de Bioquímica y Biología molecular. Facultad de Ciencias Químicas y Farmacéuticas. Universidad de Chile. [1], Laboratorio de Moléculas Individuales, Universidad Peruana Cayetano Heredia[2], Institute for Quantitative Biosciences (QB3), University of California, Berkeley, USA.[3], Jason L. Choy Laboratory of Single-Molecule Biophysics, University of California, Berkeley, USA[4]. Sponsored by Cabrera, R.

09:00 -11:00 **ORAL SESSION 4**

GENE EXPRESSION I
Salón Frutillar

Chair: Martin Montecino
Co-chair: Alejandra Loyola

09:00 **Transcriptional regulation in the 4th dimension: unravelling circadian transcriptional networks in a model eukaryote. Montenegro-Montero, A.**[1], Goity, A.[1], Weirauch, M.T.[2], Yang, A.[2], Hughes, T.R.[2], Larrondo, L.F.[1]. Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile[1], Banting and Best Department of Medical Research, Donnelly Centre for Cellular and Biomolecular Research, University of Toronto[2].

09:15 **Expression and biogenesis of mirrorRNAs in mammals. Munita, R.**[1], Parada, G.[1], Mattick, J.[2], Gysling, K.[1]. Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Chile[1], Garvan Institute of Medical Research, Australia[2]. Sponsored by Canessa, P.

09:30 **The cytosolic H3K9me1 modification occurs during the synthesis of the histone. Alvarez, F.**[1], Díaz Celis, C.[1], Ugalde, V.[1], Imhof, A.[2], Loyola, A.[1,3]. Fundación Ciencia & Vida, Santiago, Chile[1], Adolf-Butenandt-Institute, Ludwig-Maximilians-University, Munich, Germany[2], Universidad San Sebastián, Santiago, Chile[3].

09:45 **The interaction between the beneficial bacterium *Burkholderia phytofirmans* PSJN and *Arabidopsis thaliana* induces transcriptomic changes and phenotypical responses across the complete lyfe cycle of plants. Poupin, M.J.**[1], Timmermann, T.[1], Vega, A.[2], González, B.[1]. Laboratorio de Bioingeniería, Facultad de Ingeniería y Ciencias, Universidad Adolfo Ibáñez, Santiago, Chile.[1], Departamento de Ciencias Vegetales, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile. Santiago, Chile[2].

10:00 **Identification of a novel gene encoded in intron 5 of *RUNX1* gene. Hinojosa, M.**[1], Gutiérrez, S.[1]. Laboratorio de Regulación Transcripcional y Leucemia. Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción[1].

10:15 **The HIV-1 internal ribosomal entry site is active in lymphocytes. Vallejos, M.**[1], Pino, K.[1], López-Lastra, M.[1]. Laboratorio de Virología Molecular, Instituto Milenio de Inmunología e Inmunoterapia, Centro de Investigaciones Médicas, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile[1].

10:30 **Establishment of a cell culture hepatitis B virus replication system. Muñoz, F.**[1,2], Muñoz, E.[1,2], Hernández, S.[2], Venegas, M.[3], Brahm, J.[3], Gálvez, A.[4], Villanueva, R.[2], Loyola, A.[1]. Fundación Ciencia & Vida[1], Universidad Andrés Bello[2], Hospital Clínico Universidad de Chile[3], Universidad San Sebastián[4].

10:45 **RUNX1 protein auto-regulates its expression from P1 promoter. Martínez, M.**[1], Trombly, D.[2], Stein, J.[2], Stein, G.[2], Gutiérrez, S.[1]. Laboratorio de Regulación Transcripcional y Leucemia, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción[1], Department of Cell Biology and Cancer Center, University of Massachusetts Medical School, Worcester, USA[2].

11:00 -11:30 Coffe break

11:30-13:30 **SYMPOSIUM: CANCER FROM MOLECULAR INSIGHT TO POTENTIAL THERAPIES**
Salón Gran Ballroom

Chair: Andrew Quest

11:30 **Novel dimensions to epigenetic regulation in biological control and cancer. Stein, G.S.**[1], Lian, J.B.[1], Stein, J.L.[1], van Wijnen, A.J.[1]. Department of Cell Biology, University of Massachusetts Medical School, Worcester, Massachusetts[1].

12:15 **Rheb-to-AMPK in cancer: unlikely to have good intentions.** Lacher, M.[1], Campos, T.[2], Armijo, M.[2], Ziehe, J.[1], Zhu, Z.[1], Pincheira, R.[2], **Castro, A.**[2]. Helen Diller Family Comprehensive Cancer Center, University of California San Francisco, CA, USA[1], Departamento de Bioquímica y Biología Molecular, Universidad de Concepción, Chile[2].

12:40 **Tumor suppression and metastasis in melanomas and gastric cancer. Quest, A.F.**[1]. Centro de Estudios Moleculares de la Célula (CEMC), Facultad de Medicina, Universidad de Chile[1].

13:05 **Developing genetically engineered vectors to treat human gastric tumors. Montecino, M.**[1]. Center for Biomedical Research and FONDAP Center for Genome Regulation, Faculty of Biological Sciences and Faculty of Medicine, Universidad Andres Bello, Santiago, Chile[1].

13:30-15:00 Lunch

15:00 -16:30 **OSVALDO CORI CONFERENCE**
Salón Gran Ballroom

Chair: Cecilia Rojas

15:00 **Plant mitochondria: gene expression, gene transfer to the nucleus, insights into the physiological role. Jordana, X.**[1]. P. Universidad Católica de Chile, Fac. de Ciencias Biológicas, Depto. de Genética Molecular y Microbiología[1].

16:30-17:00 Coffe Break

17:00-19:00 NEW MEMBERS SESSION I

Salón Puerto Varas

Chair: Ricardo Cabrera
Co-chair: Amparo Uribe

17:00 **Energy-preserving effects of IGF-1 antagonize starvation-induced cardiac autophagy. Troncoso, R.**[1], Hill, J.A.[2], Abel, E.D.[3], LeRoith, D.[4], Lavandero, S.[1,2,5]. Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/Faculty of Medicine, University of Chile[1], University of Texas Southwestern Medical Center, Dallas, USA[2], University of Utah School of Medicine, Salt Lake City, USA[3], The Mount Sinai School of Medicine, New York, USA[4], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile[5].

17:30 **The first transmembrane domain (TM1) of beta2-subunit binds to the transmembrane domain S1 of alpha-subunit in BK potassium channels. Morera, F.J.**[1], Alioua, A.[2], Kundu, P.[2], Salazar, M.[3], González, C.[3], Martinez, A.D.[3], Stefani, E.[2], Toro, L.[2], Latorre, R.[3]. Institute of Pharmacology and Morphophysiology, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Valdivia, Chile[1], Division of Molecular Medicine, Department of Anesthesiology, University of California, Los Angeles, USA[2], Centro Interdisciplinario de Neurociencia de Valparaiso, Chile[3].

18:00 **HMGB proteins modify the activity of ATP-dependent chromatin remodeling complexes and its association to gene promoters. Hepp, M.I.**[1], Smolle, M.[2], Alarcón, V.[1], Workman, J.L.[2], Gutiérrez, J.[1]. Departamento de Bioquimica y Biologia Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile.[1], Stowers Institute for Medical Research, Kansas City, Missouri, USA.[2].

18:30 **Paleoenzymology of glucokinase/phosphofructokinase activity in the ADP-dependent sugar kinases family by resurrection of ancestral enzymes. Castro-Fernandez, V.**[1], Herrera-Morandé, A.[1], Guixé, V.[1]. Laboratorio de Bioquímica y Biología Molecular, Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile.[1].

17:00-19:00 NEW MEMBERS SESSION II

Salón Frutillar

Chair: Simon Ruiz
Co-chair: Rodrigo Gutiérrez

17:00 **Characterization of a putative grapevine Zn transporter, VvZIP3, suggests its involvement in early reproductive development in *Vitis vinifera.* L. Gainza-Cortés, F.**[1,2], Pérez-Diaz , R.[2], Pérez-Castro, R.[2,3], Tapia, J.[2], Casaretto, J.A.[2], González, S.[2], Ruiz-Lara, S.[2], González, E.[2]. Centro de Estudios Avanzados en Fruticultura[1], Universidad de Talca[2], Universidad Católica del Maule[3].

17:30 **Endocytic trafficking towards the vacuole plays a key role in the auxin receptor SCF^{TIR}-independent mechanism of lateral root formation in *A. thaliana.* Pérez-Henríquez, P.**[1,2], Raikhel, N.V.[3], Norambuena, L.[1,2]. Plant Molecular Biology Laboratory, Department of Biology, Faculty of Sciences, University of Chile, Santiago, Chile[1], Millenium Nucleus in Plant Cell Biotechnology, Chile[2], Center for Plant Cell Biology and Department of Botany and Plant Sciences, University of California, Riverside, CA 92521, USA[3].

18:00 **IRE1/bZIP60-mediated unfolded protein response plays distinct roles in plant immunity and abiotic stress responses. Moreno, A.A.**[1], Blanco, F.[1], Moreno, I.[1], Orellana, A.[1]. FONDAP Centro de Regulación del Genoma, Núcleo Milenio en Genómica Funcional de Plantas, Centro de Biotecnología Vegetal, Facultad de Ciencias Biológicas, Universidad Andrés Bello.[1]. Sponsored by Leon, G.

18:30 **The Arabidopsis JAZ2 promoter contains a G-box and thymidine-rich module that are necessary and sufficient for jasmonate-dependent activation by MYC transcription factors and repression by JAZ proteins. Figueroa, P.**[1], Browse, J.[2]. Escuela de Biotecnología, Facultad de Ciencias, Universidad Santo Tomás, Santiago, Chile[1], Washington State University, Pullman, Washington 991646340, USA[2].

19:15-20: 30 **SEVERO OCHOA CONFERENCE**

Salón Gran Ballroom

Chair: Juan Olate

19:15 **Novel concepts in the structure and dynamics of cell membranes. Goñi, F.**[1]. Unidad de Biofísica (CSIC, UPV-EHU), Universidad del País Vasco, 48940 Leioa, Spain[1].

20:30-22:00 Dinner

22:00-23:55 **POSTER SESSION I (Odd numbers)**

Salón Puerto Octay

Chair: Enrique González

1 **Effect of phorbol 12-myristate 13-acetate on a nuclear cathepsin L variant and the activation of CDP/Cux transcription factor in colon cancer cells Caco-2. Hermosilla, V.**[1], Iribarren, C.[1], Flaig, D.[1], Rivas, F.[1], Gutiérrez, S.[1], Morín, V.[1]. Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción[1].

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133 **Developing methodologies to obtain soluble nuclear matrix protein components associated with vitamin D-mediated transcriptional response in osteoblasts. Ruiz-Tagle, C.**[1], Montecino, M.[1]. Center for Biomedical Research and FONDAP Center for Genome Regulation, Faculty of Biological Sciences and Faculty of Medicine, Universidad Andres Bello, Santiago, Chile.[1].

135 **Comparisson of two funtional ammonia-lyase from Rhodobacter Sphaeroides. (Rs) Escalona, E.**[1], Guzmán, L.[2], Gentina, J.C.[3], Aguilar, F.[4], Acevedo, W.[4]. Laboratorio de química-biológica, Pontificia Universidad Católica de Valparaíso.[1], Laboratorio de química-biológica, Pontificia Universidad Católica de Valparaíso[2], Laboratorio de cultivos microbianos, Pontificia Universidad Católica de Valparaíso[3], Laboratorio de Bio-Espectroscopía Molecular, Pontificia Universidad Católica de Valparaíso[4]. Sponsored by Guzmán, L.

137 **Identification of CREB and C/EBPβ transcription factors binding elements in the proximal promoter region of human RIC-8B gene. Maureira, A.**[1,2], Sánchez, R.[1,2], Hinrichs, M.V.[1,2], Gutiérrez, J.[2,3], Olate, J.[1,2]. Laboratorio de Genética Molecular[1], Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Chile[2], Laboratorio de Regulación Transcripcional[3].

139 **Transcriptome response of *Mytilus galloprovincialis* exposed in vivo to Saxitoxin (STX) Núñez-Acuña, G.**[1], Aballay, A.E.[2], Astuya, A.P.[2], Gallardo-Escárate, C.[1]. Laboratorio de Biotecnología y Genómica Acuícola, Centro de Biotecnología, Departamento de Oceanografía, Universidad de Concepción, Casilla 160-C, Concepción, Chile.[1], Laboratorio de Genómica Marina y Cultivo Celular, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Casilla 160-C, Concepción, Chile.[2]. Sponsored by Uribe P., E.A.

141 **Analysis of non-canonical introns in the human transcriptome. Parada, G.**[1], Munita, R.[1], Gysling, K.[1]. Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Chile.[1]. Sponsored by Canessa, P.

143 **Differential regulatory role of C/EBPβ isoforms in transcriptional regulation of human albumin gene. Valenzuela, N.**[1,2], Castro, A.[2,3], Gutiérrez, J.[1,2]. Laboratorio de Regulación Transcripcional[1], Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile.[2], Laboratorio de Transducción de Señales y Cáncer[3].

145 **Gibberellin oxidase activities in *Bradyrhizobium japonicum* bacteroids. Méndez, C.**[1], Valdés, E.[1], Sequeira, G.[1], Montanares, M.[1], Rojas, M.C.[1]. Departamento de Química, Facultad de Ciencias, Universidad de Chile. [1].

147 **Ubiquitin-conjugating enzyme E2-like gene associated to pathogen response in *Concholepas concholepas*: SNP identification and transcription expression. Aguilar-Espinoza, A.**[1], Núñez-Acuña, G.[1], Chávez-Mardones, J.[1], Gallardo-Escárate, C.[1]. Laboratorio de Biotecnología y Genómica Acuícola, Departamento de Oceanografía, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción. P.O. Box 160-C. Concepción, Chile.[1]. Sponsored by Uribe P, E.A.

149 **Expression of the N-terminal half *Neisseria meningitidis* TbpB gene in *Salmonella* oral vaccines strains: immunogenicity in BALB/c mice compared to the entire TbpB gene. Touma, J.**[1], Bruce, E.[1], Ávila, A.[1], Hernández, C.[1], Venegas, A.[1]. Laboratory of Microbial Pathogenesis and Vaccine Biotechnology. Department of Molecular Genetics and Microbiology, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Portugal 49, Santiago[1].

Thursday, October 4th

09:00-11:00 SYMPOSIUM: MECHANISMS OF VASCULAR DYSFUNCTION BY ENDOTHELIAL DAMAGE
Salón Gran Ballroom

Chair: Luis Sobrevía

09:00 **Beneficial effects of L-carnitine in arterial hypertension and hypertension-related organ damage.** Mate, A.[1], Zambrano, S.[1], Blanca, A.[1], Ruiz-Armenta, M.V.[1], Miguel-Carrasco, J.L.[1], Salsoso, R.[1], Guzmán-Gutiérrez, E.[2], Pardo, F.[2], Leiva, A.[2], Sobrevía, L.[2], **Vázquez, C.M.**[1]. Department of Physiology, Faculty of Pharmacy, Universidad de Sevilla, Spain[1], Cellular and Molecular Physiology Laboratory (CMPL), Division of Obstetrics and Gynecology, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile, Chile[2].

09:30 **In utero programming of cardiovascular disease by maternal hypercholesterolemia. Palinski, W.**[1] Department of Medicine, University of California, San Diego, USA[1].

10:00 **Maternal supraphysiological hypercholesterolemia in pregnancy leads to placental endothelial dysfunction. Leiva, A.**[1], Diez de Medina, C.[1], Guzmán-Gutiérrez , E.[1], Abarzúa, F.[2], Pardo, F.[1], Sobrevía, L.[1]. Cellular and Molecular Physiology Laboratory (CMPL), Division of Obstetrics and Gynecology, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile[1], Clínica Alemana, Temuco, Chile[2].

10:30 **The role of the proline-rich tyrosine kinase PYK2 and tyrosine phosphorylation in the regulation of eNOS. Fleming, I.**[1]. Institute for Vascular Signaling, Centre for Molecular Medicine, Goethe University, Frankfurt am Main, Germany.[1].

11:00 - 11:30 Coffe break

11:30 -13:00 **PABMB CONFERENCE**

Salón Gran Ballroom

Chair: Sergio Lavandero

11:30 **PKA: Assembly of dynamic and isoform-specific macromolecular complexes. Taylor, S.**[1,2]. Department of Chemistry and Biochemistry, Pharmacology, and the Howard Hughes Medical Institute[1], University of California, San Diego, La Jolla, CA 92-93-0654[2].

13:00 - 14:30 Lunch

14:30-16:30 **ORAL SESSION 5**

GENE EXPRESSION II
Salón Puerto Varas

Chair: Paulina Bull
Co-chair: José Gutiérrez

14:30 **Epigenetic silencing of key bone phenotypic genes during neuronal development. Aguilar, R.**[1,2], Bustos, F.J.[3], Henriquez, B.[2], Rojas, A.[1,2,4,5], van Zundert, B.[2], Montecino, M.[1,2]. FONDAP Center for Genome Regulation[1], Center for Biomedical Research, Universidad Andres Bello, Santiago[2], Universidad de Concepción, Concepción[3], Universidad de Chile, Santiago, Chile[4], Pontificia Universidad Javeriana, Colombia[5].

14:45 **Contribution of arginine methyltransferases PRMT1 and PRMT4 to the vitamin D3-mediated transcriptional control in osteoblasts. Moena, D.**[1], Montecino, M.[1]. Center for Biomedical Research and FONDAP Center for Genome Regulation, Faculty of Biological Sciences and Faculty of Medicine, Universidad Andres Bello, Santiago, Chile.[1].

15:00 **Steamer is a retroelement present in the mollusk *Mya arenaria*. Arriagada, G.**[1,2], Goff, S.P.[2]. Departamento de Ciencias Biológicas, Universidad Andres Bello[1], Department of Biochemistry and Molecular Biophysics, Columbia University, USA[2].

15:15 **The mitochondrial protein MEF25 is an essential and highly specific PPR factor for RNA editing in Arabidopsis. Arenas, A.M.**[1], Moreno, S.[1], Gómez, M.I.[1], Takenaka, M.[2], Jordana, X.[1]. Departamento de Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile[1], Molekulare Botanik, Universität Ulm, Germany[2].

15:30 **Evaluating the role of the photoreceptor/GATA-type transcription factor White Collar-1 in the pathogenicity of *Botrytis cinerea*. Hevia, M.A.**[1], Canessa, P.[1], Larrondo, L.F.[1]. Depto. Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.[1].

15:45 **Study of translation initiation mechanism of full-length mRNA of the human T-cell leukemia virus type 1 (HTLV-1). Olivares, E.**[1], Rossi, F.[1], Pino, K.[1], Navarrete, C.[2], Huidobro-Toro, J.P.[2], López-Lastra, M.[1]. Laboratorio de Virología Molecular, Instituto Milenio de Inmunología e Inmunoterapia, Centro de investigaciones medicas, Escuela de Medicina, Pontificia Universidad Católica De Chile[1], Centro de Envejecimiento y Regeneración, CARE, Departamento de Fisiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile[2].

16:00 **Regulation of shoot meristem activity by the histone acetyltransferase GCN5 in *Arabidopsis thaliana*. Aquea, F.**[1,2], Long, J.[1]. Plant Molecular and Cellular Biology Laboratory. The Salk Institute for Biological Studies, La Jolla, California, USA.[1], Departamento de Genética Molecular y Microbiología. Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.[2].

16:15 **Identification of novel molecular factors affecting nitrogen use efficiency in *Arabidopsis thaliana*. Araus C.V.**[1], Vidal, E.A.[1], Puelma , T.[1], Gutiérrez, R.A.[1] Center for Genome Regulation. Millennium Nucleus Center for Plant Functional Genomics. Departamento de Genética Molecular y Microbiología. P. Universidad Católica de Chile.[1].

14:30-16:30 **ORAL SESSION 6**

MOLECULAR BIOLOGY OF THE CELL II
Salón Frutillar

Chair: Julio Tapia
Co-chair: Claudio Vásquez

14:30 **Regulation of endoplasmic reticulum-mitochondria communication by protein kinase A in stressed HeLa cells. Bravo, R.**[1], Rodríguez, A.E.[1], Quiroga, C.[1], Paredes, F.[1], Parra, V.[1], Quest, A.F.G.[1,2], Lavandero, S.[1,2,3]. Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/Faculty of Medicine, University of Chile[1], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile[2], Department of Internal Medicine, University of Texas Southwestern Medical Center, USA[3].

14:45 **Participation of VCAM-1 and insulin in the ischemic cardiomyocyte protection. García, L.**[1], Humeres, C.[1], Díaz, A.[1], Cordero, G.[1], González, V.[1], Corbalán, R.[2]. Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile[1], Facultad de Medicina, Universidad Católica de Chile[2].

15:00 **Long non-coding mitochondrial RNAs regulate Cyclin D1 and Survivin. Fitzpatrick, C.**[1,2,3], Araya, M.[1,2], Briones, M.[1,2,3], Oliveira-Cruz, L.[1,2], Burzio, L.O.[1,2,3], Burzio, V.A.[1,2,3]. Fundación Ciencia para la Vida[1], Andes Biotechnologies S.A.[2], Facultad de Ciencias Biológicas, Universidad Andres Bello[3].

15:15 **Mitochondrial dysfunction and fragmentation induced by proinflammatory cytokines in 3T3-L1 adipocytes. Kuzmicic, J.**[1,2], Hahn, W.[2], Burril, J.[2], Bernlohr, D.[2], Lavandero, S.[1,3,4]. Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/ Faculty of Medicine[1], Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, USA[2], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile[3], Department of Internal Medicine, University of Texas Southwestern Medical Center, USA[4].

15:30 **Impaired mitochondrial Ca^{2+} uptake in pathological cardiomyocyte hypertrophy disrupts the proper insulin signaling. Gutiérrez, T.**[1], Parra, V.[1], Troncoso, R.[1], Morales, P.E.[1], Contreras-Ferrat, A.[1,2], Vásquez-Trincado, C.[1], Lavandero, S.[1,2,3]. Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/Faculty of Medicine.[1], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile.[2], Department of Internal Medicine, University of Texas Southwestern Medical Center, USA[3].

15:45 **The Sall2 transcription factor is involved in p53-dependent apoptotic response to genotoxic stress. Escobar, D.**[1], Sánchez, M.F.[1], Cerro, R.P.[1], Sanhueza, D.A.[1], Castro, A.F.[1], Pincheira, R.[1]. Laboratorio de Transducción de Señales y Cáncer. Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción[1].

16:00 **Development and evaluation of oral vaccine prototypes against *Helicobacter pylori* in mice. Venegas, A.**[1], Olmos-Mejías, M.[1], Moreno, F.[1], Melo, F.[1], Mosqueira-Dinamarca, M.[1], Berkowitz, L.[1], Chamorro, N.[1], Ávila, A.[1], Bajas, F.[1], Bruce, E.[1], Hernández, C.[1], Villagrán, A.[2], Harris, P.R.[2]. Laboratory of Microbial Pathogenesis and Vaccine Biotechnology, Department of Molecular Genetics and Microbiology, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Portugal 49, Santiago[1], Gastroenterology and Nutrition Unit, Division of Pediatrics, School of Medicine, Pontificia Universidad Católica de Chile, Marcoleta 391, Santiago[2].

16:15 **Endocytosis regulation at transcriptional level in *Arabidopsis thaliana*: bZIP25 a novel functional transcription factor of endocytosis. Pizarro, L.**[1], Osorio, C.[1], Norambuena, L.[1]. Laboratorio de Biología Molecular Vegetal. Departamento de Biología. Facultad de Ciencias. Universidad de Chile[1].

16:30-17:00 Coffe break

17:00-18:55 **POSTER SESSION II (Even numbers)**
Salón Puerto Octay

Chair: Claudia Stange

2 **Expression and involvement of a cathepsin L variant in cell cycle progression of Caco-2 cells. Rivas, F.**[1], Flaig, D.[1], Perez, V.[1], Hermosilla, V.[1], Leonardi, M.[1], Puchi, M.[1], Morin, V.[1]. Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción[1].

4 **CRTC2 and BRG1 interaction is modulated by DNA damage in B cells. Zambrano, A.**[1,2], Teitell, M.[2]. Instituto de Bioquimica y Microbiologia, Facultad de Ciencias, Universidad Austral de Chile. Chile[1], Department of Pathology and Laboratory Medicine. University of California Los Angeles. USA[2]. Sponsored by Concha, I.I.

6 **Rheb regulates p27KIP through activation of AMPK. Campos, T.**[1], Palma, M.[1], Ziehe, J.[1], Pincheira, R.[1], Castro, A.F.[1]. Laboratorio de Transducción de Señales y Cáncer, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción[1].

8 **Hyperosmotic stress triggers cytoprotective autophagy in HeLa cells. Oyarzún, A.P.**[1], Troncoso, R.[1], Rodríguez, A.E.[1], Paredes, F.[1], Gatica, D.[1], Bravo, R.[1], Vasquez-Trincado, C.[1], Quiroga, C.[1], Criollo, A.[2], Lavandero, S.[1,2,3]. Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/Faculty of Medicine, University of Chile[1], Department of Internal Medicine, University of Texas Southwestern Medical Center, USA[2], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile[3].

10 **Role of Pannexin-1 in the depletion of regulatory T cells. López, X.**[1], Shoji, K.F.[2], Sáez, P.J.[2], Sáez, J.C.[2], Imarai, M.[3], Acuña-Castillo, C.[1] Centro de Biotecnología Acuícola-Universidad de Santiago de Chile[1], Departamento de Fisiología-Pontificia Universidad Católica de Chile[2], Centro de Biotecnología Acuícola- Universidad de Santiago de Chile[3].

12 **Identification of a novel Gαs phosphorylation site. Beyer, A.**[1], Pastén, P.[1], Torrejón, M.[1], Olate, J.[1], Hinrichs, M.V.[1]. Laboratorio de Genética Molecular, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Chile[1].

14 **Alteration of tumorigenic properties by knockdown of the antisense noncoding mitochondrial RNA in murine melanoma. Lobos-González, L.**[1], Silva, V.[1], Fitzpatrick, C.[1], Bendek, M.[2], Silva, V.[3], Burzio, L.O.[4], Burzio, V.[1] Andes Biotechnologies[1], Universidad Andres Bello[2], Universidad Andres Bello, Viña del Mar[3], Andes Biotechnologies, Fundacion Ciencias Para La Vida[4]. Sponsored by Burzio V.

16 **Lactate effect on mitochondria and cellular oxidative status in B16 and MDA-MB231. Ahumada, V.**[1], Ureta, A.[1], Ibañez, J.[1], Acuña-Castillo, C.[1], Miranda, D.[2], Montoya, M.[1]. Departamento de Biología. Facultad de Química y Biología. Universidad de Santiago de Chile[1], Laboratorio Inmunobioquimica. Facultad de Ciencias Químicas y Farmacéuticas. Universidad de Chile[2].

18 ***In silico* analysis of tetrahydrohyperforin suggests a potential pharmacophore for the activation of TRPC6 channels. Schüller, A.**[1,2], Montecinos-Oliva, C.[3], Parodi, J.[3], Inestrosa, N.C.[3]. Molecular Bioinformatics Laboratory, Millennium Institute on Immunology and Immunotherapy[1], Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile[2], Centro de Envejecimiento y Regeneración (CARE), Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile[3]. Sponsored by Melo, F.

20 **Inhibition of MCT1 through an adenoviral transduction of MCT1 shRNA induces a compensatory effect. Carril-Pardo, C.**[1], Elizondo-Vega, R.[1], Cortés-Campos, C.[1], Nualart, F.[2], Uribe, A.[3], García-Robles, M.A.[1]. Laboratorio de Biología Celular, Departamento de Biología Celular, Facultad de Ciencias Biológicas, Universidad de Concepción[1], laboratorio de Neurobiología y Células madres, Departamento de Biología Celular, Facultad de Ciencias Biológicas, Universidad de Concepción[2], laboratorio de Enzimología, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción[3].

22 **Breakpoints region accessibility in RUNX1 gene is independent of histone acetylation. Stuardo, M.**[1], Schnake, N.[1], Gutierrez, S.[1]. Laboratorio de Regulacion Transcripcional y Leucemia, Departamento de Bioquimica y Biologia Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción1.

24 **Transcriptional factor FoxO1 and mitochondrial metabolism in cultured cardiomyocytes. Quiroga, C.**[1], Riveros, C.A.[1], Lavandero, S.[1,2,3] Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/Faculty of Medicine, Universidad de Chile[1], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile[2], Department of Internal Medicine, University of Texas Southwestern Medical Center, USA[3].

26 **Genetic and phenotypic characterization of an *Arabidopsis* sirtuin mutant. Torres, E.**[1], Holzmann, C.[1], Montoya, P.[1], Jordana, X.[1]. Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile[1]. Sponsored by Jordana, X.

28 **Angiotensin-(1-9) counteracts mitochondrial fission induced by norepinephrine in cultured cardiomyocytes. Rivera-Mejias, P.**[1], Pennanen, C.[1], Sotomayor-Flores, C.[1], Lopez-Crisosto, C.[1], Parra, V.[1], Lavandero, S.[1,2,3]. Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/Faculty of Medicine[1], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile.[2], Department of Internal Medicine, University of Texas Southwestern Medical Center, USA[3].

30 **Unexpected antagonistic effect of gossypol and resveratrol over the viability on human leukemic cell lines. Cea, A.E.**[1], Ojeda, L.[1], Pérez, A.[1], Castillo, B.[1], Vega, E.[1], Zambrano, A.H.[1], Reyes, A.M.[1], Salas, M.R.[1]. Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile[1].

32 **Role of Sall2 transcription factor in cell proliferation and survival after acute genotoxic injury. Morales-Gedda, M.**[1], Sodir, N.[2], Nualart, F.[3], Pincheira, R.[1]. Laboratorio de Transducción de Señales y Cáncer, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.[1], Department of Pathology and Helen Diller Family Comprehensive Cancer Center, University of California San Francisco, USA.[2], Laboratorio de Neurobiología y Células Madres, Departamento de Biología Celular, Facultad de Ciencias Biológicas, Universidad de Concepción[3].

34 **Endometrial expression of progesterone receptor in subgroups of obese women with differing potential risk for developing endometrial cancer. Barahona, C.**[1], Albornoz, C.[2], Castro, F.[2], Gaete, F.[2], Peñaloza, P.[2], Celis, M.[2], Villavicencio, A.[1,3]. Instituto de Nutrición y Tecnología de los Alimentos (INTA), Universidad de Chile[1], Hospital Dr. Luis Tisné Brousse, Universidad de Chile[2], U-CANCER: Red de Medicina Traslacional en Cáncer, Universidad de Chile[3]. Sponsored by Quest, A.

36 **Recombinant expression of N-terminal end of isoform c from human Endothelin Converting Enzime-1 fused to Glutathione-S-Transferase. Niechi, I.**[1], Maldonado, E.[2], Armisén, R.[2,3], Fernández, C.[3,4], Tapia, J.C.[1,2,3]. Cell Transformation Laboratory[1], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile[2], U-CANCER: Network for Translational Medicine in Cancer, University of Chile[3], Department of Anatomopathology, HCUCH[4].

38 **mTOR pathway regulation by the TrkA neurotrophin receptor: possible implications on neuronal differentiation. Fuentes-Villalobos, F.**[1], Pincheira, R.[1], Castro, A.F.[1]. Laboratorio de Transducción de Señales y Cáncer, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción[1].

40 **Ceramides-induced insulin signaling desensitization is linked to mitochondrial network fragmentation. López-Crisosto, C.**[1], Parra, V.[1], Castro, P.[2], Lavandero, S.[1,3,4]. Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/Faculty of Medicine, University of Chile[1], Faculty of Medicine, P. Catholic University of Chile[2], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile[3], Department of Internal Medicine, University of Texas Southwestern Medical Center, USA[4].

42 **Modulation of glycogen synthesis in male germ cells treated with lithium. Salazar, E.**[1], Villarroel-Espíndola, F.[1], Angulo, C.[1], Castro, M.A.[1], Slebe, J.C.[1], Ramírez, A.[2], Concha, I.I.[1]. Instituto de Bioquímica y Microbiología, Universidad Austral de Chile[1], Instituto de Ciencia Animal, Universidad Austral de Chile[2]. Sponsored by Concha, I.I.

44 **Synergistic cytotoxic effect of resveratrol and nordihydroguaiaretic acid over human HL-60 and U937 leukemic cell lines. Parada V. D.**[1], Ojeda O., L.[1], Ferreira P., J.[2], Jaña P., F.[3], Reyes P., A.[1], Salas G., M.[1]. Instituto de Bioquimica y Microbiología, Facultad de Cs., Universidad Austral de Chile[1], Programa de Farmacología Molecular y Clínica, facultad de Medicina, Universidad de Chile[2], Programa de Farmacología Molecular y Clínica, Facultad de Medicina, Universidad de Chile[3].

46 **Development of a premium variety of apple seedlings with greater sweetness. Díaz, F.**[1], Aguayo, F.[1], Mandujano, P.[1], Zamudio, S.[1], Araya, J.[1], Arcos, Y.[2], Arce, P.[2], Norambuena, L.[1], Stange, C.[1], Handford, M.[1]. Laboratorio de Biología Molecular Vegetal, Facultad de Ciencias, Universidad de Chile[1], Laboratorio de Bioquímica, Departamento de Genética Molecular y Microbiología, P. Universidad Católica[2].

48 **Identification and characterization of pollen-specific promoters in *Arabidopsis thaliana*. Muñoz, D.**[1], León, G.[1]. Laboratorio de Reproducción y Desarrollo de Plantas, Centro de Biotecnología Vegetal, Universidad Andrés Bello[1].

50 **Functional characterization of pollen-specific kinases through the generation of knock-down transgenic plants expressing hairpin-RNAs (hpRNAs). Lucca, N.**[1], García, M.P.[1], León, G.[1] Laboratorio de Reproducción y Desarrollo de Plantas, Centro de Biotecnología Vegetal, Universidad Andrés Bello[1].

52 **Incretin GLP-1 promotes functional endoplasmic reticulum-mitochondria coupling in vascular smooth muscle cells. Morales, P.E.**[1], Torres, G.[1], Michea, L.[2], Lavandero, S.[1,2,3], Chiong, M.[1]. Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/Faculty of Medicine[1], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile[2], Department of Internal Medicine, University of Texas Southwestern Medical Center, USA[3].

54 **Antioxidant and antimicrobial capacity in leaves and fruit of peumo (*Cryptocarya alba*) and arrayan (*Luma apiculata*) tree species. Fuentes, L.**[1], Franco, W.[2], Valdenegro, M.[1], Gómez, M.G.[3], Millon, C.[3], Martínez, J.P.[4], Figueroa, C.[5]. Centro Regional de Estudios en Alimentos Saludables (CREAS), Valparaíso, Chile[1], Departamento de Ingeniería Química y Bioprocesos, Pontificia Universidad Católica de Chile, Santiago, Chile[2], Departamento de Ingeniería Química y Ambiental, Universidad Técnica Federico Santa María, Valparaíso, Chile[3], Laboratorio de Fisiología y Biología Molecular Vegetal, INIA-La Cruz, La Cruz, Chile[4], Facultad de Ciencias Forestales, Universidad de Concepción, Chile[5].

56 **Acetaldehyde as a mediator of ethanol reinforcement: Determination of acetaldehyde levels formed *in vivo* in the ventral tegmental area of rat brain following acute ethanol administration. Buscaglia, M.**[1,2], Quintanilla, M.E.[1], Rivera-Meza, M.[1], Herrera-Marschitz, M.[1,3], Morales, P.[1], Israel, Y.[1,2]. Programme of Molecular and Clinical Pharmacology, Institute of Biomedical Sciences (ICBM), Faculty of Medicine, University of Chile.[1], Department of Pharmacological and Toxicological Chemistry, Faculty of Chemical and Pharmaceutical Sciences, University of Chile.[2], Millenium Institute (BNI)-Chile.[3]. Sponsored by Sapag, A.

58 **Interaction between BIRC5 a cancer protein with SMAC mimetic peptides, a fluorescence spectroscopy approach. Carrasco, V.**[1], Guzmán, F.[1], Guzmán, L.[1], Aguilar, L.[1]. Pontificia Universidad Católica de Valparaiso[1].

60 **γ subunit of R-phycoerythrin from *Gracilaria chilensis*: sequence analysis and protein modelling. Lobos, F.**[1], Martínez-Oyanedel, J.[1], Bunster, M.[1]. Laboratorio de Biofísica Molecular, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Casilla 160-C, Concepción, Chile[1].

62 ***Helicobacter pylori* induced loss of survivin expression and gastric cell viability is linked to secretion of bacterial gamma glutamyl transpeptidase.** Valenzuela, M.[1,2], Bravo, D.[1], **Canales, J.**[1], Toledo, H.[2], Quest, A.F.[1]. Centro de Estudios Moleculares de la Célula (CEMC), Facultad de Medicina, Universidad de Chile.[1], Laboratorio de Microbiología Molecular, Programa de Biología Celular y Molecular, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile[2].

64 **Supported choice of clustering algorithms and partitions in hierarchical clustering of biological data. Slater, A.W.**[1,2], Norambuena, T.[1,2], Melo, F.[1,2]. Molecular Bioinformatics Laboratory, Millennium Institute on Immunology and Immunotherapy.[1], Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile[2].

66 **Reconstruction of the last common ancestor of the bifuncional HMPK/PLK and of the specific HMPK enzymes of ATP-dependent coenzyme kinase family. Bravo-Moraga, F.**[1], Castro-Fernández, V.[1], Ramírez-Sarmiento, C.[1], Guixé, V.[1]. Laboratorio de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Chile[1].

68 **Structural features of the NAD(P) binding sites in the SCOP Folds. Fuentealba, M.**[1], Cabrera, R.[1]. Laboratorio de Bioquímica y Biología Molecular, Departamento de Biología, Facultad de Ciencias, Universidad de Chile[1].

70 **Development of immunoassay for pathogens detection using fluorescence polarization. Silva, V.**[1], Cuevas, F.[2], Aguilar, L.F.[1]. Pontificia Universidad Católica de Valparaíso[1], Universidad Santo Tomas[2]. Sponsored by Guzmán, L.

72 **Comparative modeling of the TraY DNA binding protein reveal a trefoil knot topology. Molina, J.**[1], Floor, M.[1], Reyes, J.[1], Bustamante, A.[1], Baez, M.[1]. Laboratorio de Bioquímica, Departamento de Bioquímica y Biología molecular. Facultad de Ciencias Químicas y Farmacéuticas. Universidad de Chile[1]. Sponsored by Cabrera, R.

74 **Characterization of the role of residue Y671 in the ionic conductance of the TRPV1 channel. Vásquez, Y.**[1], Poblete, H.[1], Brauchi, S.[2], González-Nilo, D.[3]. Centro de Bioinformática y Simulación Molecular, Universidad de Talca.[1], Centro Interdisciplinario de Neurociencias de Valparaiso, Facultad de Ciencias, Universidad de Valparaiso.[2], Centro de Bioinformática y Biología Integrativa, Universidad Andrés Bello.[3]. Sponsored by González, W.

76 **FragProt, a webserver and database of protein fragments clustered by structural similarity. Rodríguez, F.**[1,2], Slater, A.[1,2], Melo, F.[1,2]. Molecular Bioinformatics Laboratory, Millennium Institute on Immunology and Immunotherapy[1], Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile[2].

78 **Structure determination in the crystalline state and in solution reveals a conserved mechanism for catalysis in hiperthermophilic ADP-dependent glucokinases. Herrera-Morandé, A.**[1,2], Rivas-Pardo, J.A.[1], Castro-Fernández, V.[1], Fernández, F.J.[2], Vega, M.[2], Guixé, V.[1]. Departamento de Biología, Facultad de Ciencias, Universidad de Chile [1], Centro de Investigaciones Biológicas-CSIC, Madrid, España[2].

80 **Effects of monovalent cations on the allosteric MgATP inhibition of phosphofructokinase-2 from *Escherichia coli*. Blanco, A.**[1,2], Villalobos, P.[1], Baez, M.[3], Babul, J.[1]. Departamento de Biología, Facultad de Ciencias, Universidad de Chile.[1], Escuela de Ingeniería en Bioinformática, Universidad de Talca.[2], Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.[3].

82 **Estimation of binding free energy of antagonists against different subtypes of nAChRs: effect of clustering in the application of the MM-GBSA method. Pareja, C.**[1], Adasme, F.[1], Iturriaga-Vásquez, P.[2], Alzate-Morales, J.[1]. Centro de Bioinformática y Simulación Molecular (CBSM), Escuela de Ingeniería, Universidad de Talca, 2 Norte 685, Casilla 721, Talca, Chile.[1], Laboratorio de Química Biodinámica, Departamento de Química, Facultad de Ciencias, Universidad de Chile, Las Palmeras 3425, Santiago, Chile[2]. Sponsored by González, W.

84 **Molecular Characterization of Shaker Potassium Channel Inactivation. Vergara-Jaque, A.**[1], Poblete, H.[1], Holmgren, M.[2]. Centro de Bioinformática y Simulación Molecular. Universidad de Talca.[1], National Institute of Neurological Disorders and Stroke, National Institutes of Health. USA.[2]. Sponsored by González, W.

86 **Structural determinants of nucleotide binding site in ribokinase of *Homo sapiens*. Leon, M.**[1], Guixé, V.[1]. Laboratorio de Bioquímica y Biología Molecular, Departamento de Biología, Facultad de Ciencias, Universidad de Chile[1].

88 **Functional analyses of HCV RNA polymerase mutants in potential phosphorylation sites at the fingers subdomain, and profile of posttranslational modifications of the recombinant protein. Hernández, S.**[1], Muñoz, E.[2], Díaz, A.[1], Loyola, A.[2], Villanueva, R.A.[1]. Departamento de Ciencias Biológicas, Universidad Andres Bello.[1], Fundacion Ciencia & Vida[2].

90 **Hydrogen peroxide affects the activity and thermal stability of Glutamyl-tRNA Reductase from *Acidithiobacillus ferrooxidans*. Farah, C.**[1], Ibba, M.[2], Orellana, O.[1]. Programa de Biología Celular y Molecular, Facultad de Medicina, Universidad de Chile [1], Microbiology Department, The Ohio State University [2].

92 **Evaluation in silico of the copper-binding ability of a Kunitz trypsin inhibitor in poplars (*Populus* spp). Valenzuela Riffo, F.**[1], Guerra, F.[2]. Escuela de Ingeniería en Bioinformática, Universidad de Talca[1], Instituto de Biología Vegetal y Biotecnología, Universidad de Talca[2]. Sponsored by Martínez Oyanedel, J.

94 **Molecular modeling of NAC42 transcription factor and the binding to specific cis-regulatory elements. Soto, F.**[1], Caballero, J.[1], González, W.[1]. Universidad de Talca[1]

96 **The deletion of 38 amino acids of the C-terminal and the sited-directed mutagenesis of Ala19Tyr decrease the Feroxidase activity but improve the formation of a homogeneous iron core in *Cholorobium tepidum* Ferritin. Yévenes, A.**[1], Brito, C.[2], Márquez, V.[2], Sandoval, C.[2], González-Nilo, F.[2], López-Castro, J.[3], Domínguez-Vera, J.M.[4], Watt, R.[5]. Pontificia Universidad Católica de Chile[1], Universidad Andrés Bello[2], Universidad de Cadiz[3], Universidad de Granada[4], Brigham Young University[5].

98 **Molecular dynamics simulations of the binding of phosphorylcholine (Pch) to phosphorylcholine phosphatase (PchP). Bustos, D.**[1], Vergara-Jaque, A.[1], Beassoni, P.[2], Alzate-Morales, J.[1]. Centro de Bioinformática y Simulación Molecular. Universidad de Talca.[1], Departamento de Biología Molecular, Universidad Nacional Rio Cuarto, Córdoba, Argentina.[2]. Sponsored by González, W.

100 **Heterologous expression of a *Penicillium purpurogenum* beta-D-xylosidase in *Pichia pastoris* and its characterization. Ravanal, M.C.**[1], Eyzaguirre, J.[1]. Universidad Andrés Bello[1].

102 **Sequencing and characterization of a recombinant endopolygalacturonase of *Aspergillus fumigatus* heterologously expressed in *Pichia pastoris* GS115. Pérez, N.**[1], Eyzaguirre, J.[1]. Universidad Andrés Bello[1].

104 **Docking and biological studies of coumarins derivatives as lipoxygenase inhibitors. Silva, F.**[1], Núñez, C.[1], Mascayano, C.[1], García-Beltrán, O.[2], Cassels, B.[2], Fierro, A.[3]. Departamento de Ciencias del Ambiente, Facultad de Química y Biología, Universidad de Santiago de Chile[1], Departamento de Química, Facultad de Ciencias, Universidad de Chile, Santiago, Chile[2], Departamento de Química Orgánica, Facultad de Química, Universidad Católica de Chile, Santiago, Chile[3]. Sponsored by González-Nilo, D.

106 **The differential expression profile of CXCR3 receptor spliced variants could contribute to malignant phenotype observed in papillary thyroid cancer. Fischer, M.**[1], Urra, S.[1], Martínez., R.[1], Catalán, T.[2], Vargas, S.[1], Véliz. , L.[1], Kalergis, A.[3,4,5], Riedel, C.[6], González, H.[1]. Departamento de Cirugía Oncológica, Facultad de Medicina, Pontificia Universidad Católica, Santiago, Chile[1], Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontifica Universidad Católica, Santiago, Chile[2], Millennium Institute on Immunology and Immunotherapy, Santiago, Chile.[3], Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontifica Universidad Católica, Santiago, Chile.[4], Departamento de Reumatología, Facultad de Medicina, Pontificia Universidad Católica, Santiago, Chile[5], Facultad de Ciencias Biológicas, Universidad Andrés Bello, Santiago, Chile[6].

108 **Evolutionary history of the *SPARC* gene: ancestral functions and recruitment into the vertebrate skeletal cell genetic program. Hanna, P.**[1], Bertin, A.[1], Aldea, D.[1], Otárola, G.[1], Muñoz, D.[1], Sachs, L.[2], Buisine, N.[2], Hitoyoshi, Y.[3], Hudson, C.[3], Fuentealba, J.[1], Torrejón, M.[1], Escrivá, H.[3], Bertrand, S.[3], Marcellini, S.[1]. Faculty of Biological Sciences, University of Concepción, Chile[1], Muséum National d'Histoire Naturelle, CNRS, France[2], Université Pierre et Marie Curie, CNRS, France[3]. Sponsored by Gutiérrez, L.

110 **Insulin-increased human equilibrative nucleoside transporter 2 involves Srebp-1c increased expression in human umbilical vein endothelial cells. Pardo, F.**[1], Westermeier, F.[1], Salomon, C.[1], Guzmán-Gutiérrez , E.[1], Arroyo, P.[1], Santos, M.[1], Leiva, A.[1], Sobrevía, L.[1]. Cellular and Molecular Physiology Laboratory (CMPL), Division of Obstetrics and Gynecology, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile [1].

112 **The role of Ca^{2+} in the nitrate signaling pathway in Arabidopsis thaliana roots. Riveras, E.**[1], Oses, C.[1], Tamayo, K.[1], Gutiérrez, R.[1]. Center for Genome Regulation. Millennium Nucleus Center for Plant Functional Genomics. Departamento de Genética Molecular y Microbiología. P. Universidad Católica de Chile[1].

114 **The NE1 scaffold protein regulates Arabidopsis nitrogen use efficiency and plant growth. Vidal, E.A.**[1,2,3], Araus, V.[1,2,3], Puelma, T.[1,2,3], Gutiérrez, R.A.[1,2,3]. FONDAP Center for Genome Regulation[1], Millennium Nucleus Center for Plant Functional Genomics[2], Departamento de Genetica Molecular y Microbiologia, Pontificia Universidad Catolica de Chile[3].

116 **Expression profiles of members from a gene family coding for zinc-uptake transporters in grapevine. González, S.**[1], Gaínza-Cortés, F.[2], Pérez-Díaz, R.[1], Ruiz-Lara, S.[1], González, E.[1]. Instituto de Biología Vegetal y Biotecnología, Universidad de Talca[1], Centro de Estudios Avanzados en Fruticultura (CEAF), Conicyt Regional R08I1001[2].

118 **UV-induced transcriptomic response of human keratinocytes treated with aqueous extract of *Deschampsia antarctica*. García, H.**[1], Bizama, C.[1], Osorio, J.[1], Gutiérrez, A.[1], Gidekel, M.[1]. Uxmal S.A[1]. Sponsored by Ana, G.

120 **Cytokinin signaling and nitrate induced root growth in Arabidopsis thaliana.** Naulin, P.[1], **Tamayo, K.**[1], Gras, D.[1], Vega, A.[1], De la Cruz, J.[1], Gutiérrez, R.[1]. Center for Genome Regulation. Millennium Nucleus Center for Plant Functional Genomics. Departamento de Genética Molecular y Microbiología. P. Universidad Católica de Chile[1].

122 **Global Search of Novel Transcriptional Regulators involved in the *Neurospora crassa* Circadian Clock. Muñoz-Guzmán, F.**[1], Caballero, V.[1], Larrondo, L.F.[1]. Depto. Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile[1].

124 **Evolution of the roles of the canonical Wnt and BMP pathways during vertebrate osteogenesis: lessons from the amphibian *Xenopus tropicalis*. Bertin, A.**[1], Hanna, P.[1], Aldea, D.[1], Otárola, G.[1], Muñoz, D.[1], Sachs , L.[2], Buisine, N.[2], Henríquez, J.P.[1], Marcellini, S.[1]. Faculty of Biological Sciences, University of Concepción, Chile[1], Muséum National d'Histoire Naturelle,CNRS, France[2]. Sponsored by Gutiérrez, J.L.

126 **Effect of oxidative stress on the vernalization response using several *Arabidopsis* ecotypes that express the *FRI/FLC* module. Moraga, F.**[1], León, G.[1]. Laboratorio de Desarrollo y Reproducción de Plantas, Centro de Biotecnología Vegetal, Universidad Andrés Bello [1].

128 **Characterization of promoters from grapevine with different expression profiles to develop cisgenic grapes. Espinoza, C.**[1], Fredes, I.[1], Herrera, D.[1], Torres, E.[2], Arce, A.[1], Serrano, A.[1], Larraín, R.[1], Marchandón, G.[3], Medina, C.[1], Arce-Johnson, P.[1]. Departamento de Genética Molecular y Microbiología. Facultad de Ciencias Biológicas. Pontificia Universidad Católica de Chile, Santiago, Chile.[1], Facultad de Ciencias. Universidad de Chile, Santiago, Chile.[2], Facultad de Ingeniería. Universidad de Talca, Talca, Chile[3].

130 **Unraveling the spinal cord regeneration transcriptome of *Xenopus laevis*. Almonacid, L.I.**[1,2], Lee-Liu, D.[3,4], Moreno, M.[3], Melo, F.[1,2], Larrain, J.[3]. Molecular Bioinformatics Laboratory, Millennium Institute on Immunology and Immunotherapy[1], Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Alameda 340, Santiago, Chile[2], Center for Aging and Regeneration and Millennium Nucleus in regenerative Biology[3], Faculty of Chemical and Pharmaceutical Sciences, Universidad de Chile, Santiago, Chile[4].

132 **Ferritin characterization and identification of single nucleotide polymorphism (SNP) associated with innate immune response *Concholepas concholepas*. Chávez - Mardones, J.**[1], Nuñez - Acuña, G.[1], Maldonado - Aguayo , W.[1], Valenzuela - Muñoz, V.[1], Gallardo - Escárate, C.[1]. Laboratorio de Biotecnología y Genómica Acuícola, Departamento de Oceanografía, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción. P.O. Box 160-C. Concepción, Chile.[1]. Sponsored by Uribe P., E.A.

134 **HMGA1a protein positively stimulates the binding of estrogen receptor and its effect on the chromatin remodeling complex SWI/SNF. Del Rio, V.**[1], Hepp, M.I.[1], Gutiérrez, J.[1]. Laboratorio de Regulación Transcripcional, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.[1].

136 **Similar states of DNA methylation in MLH1 gene are associated with different patterns of histone modifications in different types of cancer cells. Sepúlveda, J.H.**[1], Gutiérrez, S.E.[1]. Laboratorio de Regulación Transcripcional y Leucemia, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción[1].

138 **Contribution of sumoylation during regulation of vitamin D-mediated transcription in osteoblastic cells. Merino, P.**[1], Montecino, M.[1]. Center for Biomedical Research and FONDAP Center for Genome Regulation, Faculty of Biological Sciences and Faculty of Medicine, Universidad Andres Bello, Santiago, Chile[1].

140 **Mining single nucleotide polymorphism (SNP) in *Haliotis rufescens* using transcriptome pyrosequencing data. Valenzuela-Muñoz, V.**[1], Uribe, D.[1], Gallardo-Escárate, C.[1]. Laboratorio de Biotecnología y Genómica Acuícola, Centro de Biotecnología. Departamento de Oceanografia. Universidad de Concepción, Concepción, Chile. [1]. Sponsored by Uribe P., E.A.

142 **Evaluation of the methylation status of RPRM gene in gastric cancer models. Marchant, M.J.**[1,2], Corvalán , A.[2], Guzmán, L.[1]. Instituto de Química, Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso.[1], Centro de Investigaciones Médicas, Pontificia Universidad Católica de Chile[2].

144 **Effects of early CPPU treatments in the gene expression of putative cyclins and berry size in *Vitis vinifera*. Jáuregui R., F.**[1], Pérez-Donoso, A.[1]. Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile[1]. Sponsored by Vega, A.

146 **Molecular characterization of two Serine Proteinase Inhibitor genes in the surf clam *Mesodesma donacium* exposed to *Vibrio anguillarum*. Maldonado-Aguayo, W.**[1], Chávez-Mardones, J.[1], Núñez-Acuña, G.[1], Valenzuela-Muñoz, V.[1], Gallardo-Escárate, C.[1]. Laboratorio de Biotecnología y Genómica Acuícola, Departamento de Oceanografía, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción. P.O. Box 160-C. Concepción, Chile.[1]. Sponsored by Uribe P, E.A.

148 **Functional studies of the PcAce1 transcriptional factor from the fungus *Phanerochaete chrysosporium* by copper. Silva, M.**[1], Essus, K.[1], Riffo, M.[1], Bull, P.[1]. Pontificia Universidad Católica de Chile[1].

09:00-20:15 **SCIENTIFIC DIALOGUES**

Salón Gran Ball Room

Chair: Eduardo Kessi

19:00 **Living in a seismic country: advantages and disadvantages. Comte, D.**[1]. AMTC-Facultad de Ciencias Físicas y Matemáticas, Universidad de Chile[1].

20:15-21:00 **SBBM MEMBERS MEETING**

21:30 Dinner

Friday, October 5th

10:00-12:00 NEW MEMBERS SESSION III
Salón Puerto Varas

Chair: Ilona Concha
Co-chair: Ricardo Cabrera

10:00 **Glycosylations in the globular head of the hemagglutinin protein modulate the virulence and antigenic properties of the H1N1 influenza viruses. Medina, R.A.**[1,2,3], Stertz, S.[2,3,4], Manicassamy, B.[2,3], Sun, X.[5], Albrecht, R.A.[2,3], Belshe, R.B.[6], Frey, S.E.[6], Tumpey, T.M.[5], García-Sastre, A.[2,3,7]. Laboratory of Molecular Virology, Instituto Milenio en Inmunología e Inmunoterapia, Centro de Investigaciones Médicas y División de Pediatría, Facultad de Medicina, Pontificia Universidad Católica de Chile[1], Department of Microbiology[2], Global Health and Emerging Pathogens Institute, Mount Sinai School of Medicine, New York, USA[3], Institute of Medical Virology, University of Zurich, Zurich, Switzerland[4], Influenza Division, National Center for Immunization and Respiratory Disease, Centers for Disease Control and Prevention, Atlanta, USA[5], Saint Louis University School of Medicine, Saint Louis University, St. Louis, USA[6], Department of Medicine, Division of Infectious Diseases, Mount Sinai School of Medicine, New York, USA[7]. Sponsored by López-Lastra, M.

10:30 **Insulin-dependent GLUT4 translocation requires concerted NADPH oxidase-RyR1 axis and IP3R activation in skeletal muscle cells. Contreras-Ferrat, A.**[1], Klip, A.[2], Lavandero, S.[1,3], Jaimovich, E.[1]. Center for Molecular Studies of the Cell and Biomedical Sciences Institute, Faculty of Medicine, Universidad de Chile[1], Cell Biology Program, The Hospital for Sick Children, Toronto, Ontario, Canada M5G [2], Department of Internal Medicine (Cardiology), University of Texas Southwestern Medical Center, Dallas, Texas 75235, USA[3].

11:00 **Herp depletion protects from protein aggregation by up-regulation of Atg5 and Beclin-1 during early stress conditions. Quiroga, C.**[1], Gatica, D.[1], Paredes, F.[1], Bravo, R.[1], Troncoso, R.[1], Pedrozo, Z.[1,2], Rodríguez, A.E.[1], Vicencio, J.M.[3], Chiong, M.[1], Hetz, C.[1,2], Lavandero, S.[1,2,4]. Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/ Faculty of Medicine, Universidad de Chile[1], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile[2], Department of Cell and Developmental Biology and Consortium for Mitochondrial Research, University College London, United Kingdom[3], Department of Internal Medicine, University of Texas Southwestern Medical Center, USA[4].

11:30 **Muscle glycogen synthase isoform is responsible for testicular glycogen synthesis: glycogen overproduction induces apoptosis in male germ cells. Villarroel-Espíndola, F.**[1], Mancilla, H.[1], Vander Stelt, K.[1], Maldonado, R.[1], Acuña, A.[1], Covarrubias, A.[1], López, C.[1], Angulo, C.[2], Castro, M.[1], Slebe, J.C.[1], Durán, J.[3], Rocha-García, M.[3], Guinovart, J.J.[3], Concha, I.I.[1]. Instituto de Bioquímica y Microbiología, Universidad Austral de Chile, Valdivia, Chile[1], Instituto de Química, Universidad Austral de Chile, Valdivia, Chile[2], Institute for Biomedical Research (IRB), Barcelona, España[3].

10:00-12:00 NEW MEMBERS SESSION IV
Salón Frutillar

Chair: Juan Olate
Co-chair: Ariel Castro

10:00 **Response to inclination in young seedlings of radiata pine. Hormonal signaling and molecular players. Ramos , P.**[1], Valenzuela, C.[1], Cruz, N.[1], Moya-León, M.A.[1], Herrera, R.[1]. Laboratorio de Fisiología Vegetal y Genética Molecular, IBVB, Universidad de Talca, Chile[1].

10:30 **Transcriptome analyses identifies specific intracellular pathways and target genes in gastric cancer. Bizama, C.**[1], Felipe, B.[2], Jaime , E.[2], Salvatierra, E.[3], Ana, G.[2], Elmer, F.[4], Eduardo, S.[5], Iván , R.[6], Guillermo , M.[7], Osvaldo , P.[3]. Programa de Doctorado en Ciencias Mención Biología Celular y Molecular Aplicada. Facultad de Ciencias Agropecuarias y Forestales. Universidad de La Frontera, Temuco, Chile. [1], Programa de Doctorado en Ciencias Mención Biología Celular y Molecular Aplicada. Facultad de Ciencias Agropecuarias y Forestales. Universidad de La Frontera, Temuco, Chile[2], Laboratorio de Terapia Celular y Molecular. Fundación Instituto Leloir, Buenos Aires, Argentina. [3], Facultad de Ingeniería, Grupo de Minería de Bio-Datos. Universidad Católica de Córdoba, Argentina.[4], Biotecnología. Facultad de Ciencias Agropecuarias y Forestales. Universidad de La Frontera, Temuco, Chile.[5], Servicio de Patología, Clínica Alemana de Santiago. Facultad de Medicina, Universidad del Desarrollo, Chile.[6], Laboratorio de Terapia Génica. Escuela de Medicina. Universidad Austral. Pilar-Buenos Aires, Argentina.[7] Sponsored by Gutiérrez, A.

11:00 **Structural characterization and substrate specificity of VpAAT1 protein related to ester biosynthesis in mountain papaya fruit. Morales-Quintana, L.**[1], Moya-León, M.A.[1], Herrera, R.[1]. Laboratorio de Fisiología Vegetal y Genética Molecular, Instituto de Biología Vegetal y Biotecnología, Universidad de Talca[1].

11:30 **TMBIM3/GRINA is a novel unfolded protein response (UPR) target gene that controls apoptosis through the modulation of ER calcium homeostasis. Rojas-Rivera, D.**[1,2,3], Armisen, R.[2], Colombo, A.[1], Martínez, G.[1,2,3], Eguiguren, A.L.[2], Díaz, A.[1], Kiviluoto, S.[4], Rodríguez, D.[1,2,3], Patron, M.[5], Rizzuto, R.[5], Bultynck, G.[4], Concha, M.[1], Sierralta , J.[1], Stutzin, A.[2], Hetz, C.[1,2,3]. Biomedical Neuroscience Institute, Faculty of Medicine, University of Chile[1], Center for Molecular Studies of the Cell, Institute of Biomedical Sciences, University of Chile, Santiago, Chile[2], Neurounion Biomedical Foundation, Santiago, Chile[3], Katholieke Universiteit Leuven[4], Universita degli Studi di Padova[5]. Sponsored by Lavandero, S.

12:00 - 12:15 Coffe break

12:15 - 12: 45 **Herman Niemeyer Medal Award (Chair: Ilona Concha)**

12:45 -13:00 **Best Oral, Poster and New Members Awards (Chair: Lorena Norambuena)**

13:00 -13:30 **Tito Ureta Award (Chair: Victoria Guixé)**

CONFERENCES

Modelling new strategies to treat cancer. Evan, G.I.[1] Dept. Biochemistry, Sanger building, 80 Tennis Court Rd, Cambridge CB2 1GA, UK[1]. Despite dramatic advances in the use of conventional chemo and radiotherapy and the growth of more "targeted" drugs, cancers remain lethal disease in desperate need of new therapeutic approaches. The principal problem is that we have no systematic or informed way of predicting which, out of the legion of aberrant processes in cancer cells, is the best to target with drugs or where, within that process, the most effective molecular targets lie. As a consequence, drug development is driven by what is deemed feasible and deliverable rather than by what might be most therapeutically effective. To address this problem, we employ a novel class of genetically engineered mouse (GEM) in which individual oncogenes and/or tumor suppressor genes may be systemically toggled off and on, reversibly and at will, in vivo. In this way we can test and validate therapeutic targets irrespective of contemporary (and ephemeral) prejudices as to their "druggability" . This approach will be illustrated by three such GEMs: one mouse that models pharmacological inhibition of Myc, a core component of the replicative machinery of all tumour and normal cells and a downstream conduit for many (perhaps all) oncogenic growth signals, a second mouse that models inhibition of E2F, the transcription factor required to drive cells into the cell cycle, and a third that models pharmacological restoration of the p53 tumour suppressor that is functionally inactivated in most human cancers. Since it is the endogenous oncogene or tumour suppressor that is regulatable in such GEMs, they can be easily applied to any existing or new preclinical mouse cancer model. Using these three GEMs, we have directly ascertained the therapeutic impact, efficacy and side effects of Myc and E2F inhibition and p53 restoration - establishing mechanism of action, side effects on normal tissues and therapeutic efficacy against different types of cancer.

Plant mitochondria: gene expression, gene transfer to the nucleus, insights into the physiological role. Jordana, X.[1]. P. Universidad Católica de Chile, Fac. de Ciencias Biológicas, Depto de Genética Molecular y Microbiología[1].
Plant mitochondria share essential functions (i.e. oxidative phosphorylation) with those of other eukaryotes. However, they carry additional metabolic pathways which are not present for instance in animal mitochondria and are probably related to autotrophy. Mitochondrial biogenesis and function depends on mitochondrial genome expression, which is complex and includes post-transcriptional steps such as RNA editing. We have contributed to establish the essential role of RNA editing for proper mitochondrial gene expression and have recently identified specificity factors for several editing sites. During evolution most of the genes encoding mitochondrial proteins have been transferred to the nucleus. This gene transfer is still underway in higher plants and we have characterized some mechanisms of nuclear adaptation (acquisition of promoter, targeting peptide) using ribosomal protein genes as models. To analyze mitochondrial function we have characterized Arabidopsis mutants in respiratory complex II (succinate dehydrogenase, SDH) because of its essential role in both the electron transport chain and the tricarboxylic acid cycle. Our work on a flavoprotein subunit mutant revealed a role of complex II (and mitochondria) in gametophyte development, and in improving leaf photosynthesis. Characterization of a null mutant for one of the three genes encoding the iron-sulfur subunit revealed a role for SDH in germination, hypocotyl elongation and seedling establishment. This gene is specifically expressed in the embryo during seed maturation, being regulated by B3 domain and bZIP transcription factors.Supported by Fondecyt 1100601 and Millennium Nucleus in Plant Functional Genomics (P10-062-F).

Novel concepts in the structure and dynamics of cell membranes. Goñi, F.[1]. Unidad de Biofísica (CSIC, UPV-EHU), Universidad del País Vasco, 48940 Leioa, Spain[1].
The proposal in 1972 of a "fluid mosaic model" for the structure of biomembranes, by Singer and Nicholson, made such an impact in the field that even now it is considered as the standard model for the interpretation of most biochemical and biophysical studies on membranes. However, significant progress has occurred in the forty years elapsed so that the model, while remaining essentially unchallenged, must incorporate a number of well-accepted new data. This talk will review these novel aspects, that can be summarized as: (i) the density of transmembrane protein domains in the bilayer is very high, thus there is very little unperturbed lipid; (ii) the membrane is laterally heterogeneous, with micro- and perhaps nanodomains with specific protein and lipid composition exerting unique physiological roles; (iii) under certain conditions, membrane lipids can adopt non-bilayer structures, in a localized, transient way, and (iv) in some situations transbilayer ("flip-flop") lipid motion can take place, with important functional consequences, e.g. apoptosis.

PKA: Assembly of dynamic and isoform-specific macromolecular complexes. Taylor, S.[1,2]. Department of Chemistry and Biochemistry, Pharmacology, and the Howard Hughes Medical Institute[1], University of California, San Diego, La Jolla, CA 92-93-0654[2].
cAMP-dependent protein kinase (PKA) serves as a prototype for the entire superfamily. In contrast to metabolic enzymes, which have evolved to be efficient catalysts, protein kinases have evolved to be molecular switches, similar to the G Proteins, using a variety of mechanisms to regulate the active enzyme. Typically the linkers, loops, and inserts mediate this dynamic regulation. Phosphorylation of the Activation Loop is a common mechanism for activation, and we show how addition of a single phosphate can significantly enhance the stability, structure and kinetic properties of the C-subunit. In cells the C-subunit is packaged as holoenzymes with regulatory (R) subunits so that the activity of PKA is fully dependent on cAMP. The four functionally non-redundant R-subunits (RIα, RIβ, RIIα, and RIIβ) all have a conserved domain organization with a dimerization/ docking (D/D) domain at the N-terminus followed by a flexible linker that contains an inhibitor site and finally two tandem C-terminal cyclic nucleotide binding (CNB) domains. Specificity is determined by the isoform diversity of the R-subunits and by targeting, which is typically mediated by A Kinase Anchoring Proteins (AKAPs), which bind through an amphipathic helix to the D/D domains of the R-subunits. Specific PKA holoenzymes are then localized in close proximity to dedicated substrates. The functional non-redundancy of the R-subunits was not understood in molecular terms until structures of tetrameric holoenzymes were solved. The quaternary structures of each R:C tetramer are different and define distinct mechanisms for allosteric regulation

Living in a seismic country: advantages and disadvantages. **Comte, D.**[1]. AMTC-Facultad de Ciencias Físicas y Matemáticas, Universidad de Chile[1].

The national and international scientific community recognizes the western coast of South America as one of the most seismically active boundaries in the world. Although it is now well established that the subduction of the Nazca plate beneath the South American continental plate is the main cause of this seismic activity, associated to several seismic sources, which are located both along the oceanic subducting plate, as well in the upper continental plate. Thus, major seismic sources in Chile are interplate, intraplate deep, and shallow earthquakes, having different rates of seismicity. From the late XVI century, there have been about one earthquake above magnitude 8 every 10 years throughout our country, and any coastal area is exposed to experience an earthquake of such magnitude in 100 years. All of them have generated significant human and economic losses, making the levels of exposure and vulnerability to the effects of earthquakes in Chile are very high. However, this actually represents a great opportunity from the point of view of personal and society development. Moreover, this permanent source of energy allows us to understand sub-soil processes, which have interesting applications in mineral exploration and geothermal fields. We are proud of our beautiful geography, from coast to mountains, and it is important to point out that it exists because of the subduction process, therefore, when we learn about how to live with earthquakes we are also learning to value them from a global perspective.

SYMPOSIA

Systems approaches to unravel plant gene regulatory networks. Grotewold, E.[1]. Center for Applied Plant Sciences, The Ohio State University, Columbus, OH 43210.[1].

Our long-term goal is to investigate the mechanisms by which plants control gene expression and to elucidate the structure and dynamics of the underlying gene regulatory networks (GRNs). We have used a number of cellular processes to explore the architecture of plant GRNs in plant model systems such as maize) and Arabidopsis. For example, studies on the regulation of maize flavonoid pigments established new aspects of MYB/bHLH combinatorial gene regulation and identified previously unknown branches of the pathway. The differentiation of Arabidopsis epidermal cells into leaf hairs and pores (stomates) furnished an outstanding system for the application of systems approaches to understanding GRN architecture, which combined with modeling is providing novel insights on sub-network dynamics. The development of two public databases, AGRIS (http://arabidopsis.med.ohio-state.edu/) for *Arabidopsis* and GRASSIUS (www.grassius.org) for maize and other grasses, information on transcription factors, promoters and their interactions is integrated, significantly facilitates the identification and visualization of GRNs.

Mapping plant hormone signaling networks using halo-tag protein arrays. Yazaki, J.[1,2], Galli, M.[2], Kim, A.Y.[2], Nito, K.[1], Guillen, F.A.[3], Carvunis, A.[4,5], Chang , K.N.[1], Quan , R.[2], Nguyen, H.[2], Song, L.[1], Alvarez, J.M.[6], Gutiérrez , R.A.[6], Schroeder, J.I.[3], Chory, J.[1,7], Ramachandran, N.[8], Braun , P.[9], LaBaer, J.[10], Vidal, M.[4,5], Ecker, J.R.[1,2,7]. Plant Biology Laboratory, The Salk Institute for Biological Studies, 10010 North Torrey Pines Road La Jolla, CA 92037, USA. [1], Genomic Analysis Laboratory, Salk Institute for Biological Studies, La Jolla, CA 92037, USA[2], Division of Biological Sciences, Cell and Developmental Biology Section, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0116, USA[3], Center for Cancer Systems Biology and Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, MA 02215, USA,[4], Department of Genetics, Harvard Medical School, Boston, MA 02115, USA[5], FONDAP Center for Genome Regulation, Millennium Nucleus Center for Plant Functional Genomics, Departamento de Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile, Alameda 340, Santiago, Chile[6], Howard Hughes Medical Institute, The Salk Institute for Biological Studies, 10010 North Torrey Pines Road La Jolla, CA 92037.[7], Life Technologies Corporation, Carlsbad, CA 92008, USA.[8], Center of Life and Food Sciences, Weihenstephan der Technischen Universitaet Muenchen, Alte Akademie 8 85354 Freising, Germany.[9], The Biodesign Institute, Personalized Diagnostics, Arizona State University, Tempe, AZ 85287, USA.[10].

Protein microarrays are unparalleled in their capacity to investigate diverse biochemical properties for thousands of proteins in a single experiment. Using a novel high-affinity protein microarray approach termed HALO-tag nucleic acid programmable protein assay (HT-NAPPA), we created high-density arrays comprising 12,000 *Arabidopsis* ORFs and used these to query protein-protein interactions for a set of 38 transcription factors from a diverse set of plant hormone regulatory pathways. The resulting transcription factor interactome network, TFNAPPA, contains thousands of novel interactions. Evaluation of the quality of TFNAPPA using pulldown assays revealed that 65% of interactions were reproducible. We observed 7 pairs of co-localizations and biophysical interactions using BiFC assays by testing a set of twenty six. The novel TFNAPPA transcription factor interactome network will provide the foundation to expand our knowledge of plant hormone signaling pathways.HT-NAPPA technology can easily be applied to other organisms, e.g. mammalian model systems for which large ORF clone collections already exist. In addition to detecting protein-protein interactions, this proteome-scale system might also be utilized to expand to other applications including the identification of small molecule- protein interactions, and post-translational modifications (modifiome) such as phosphorylation, ubiquitination, methylation.

Applications of metabolomics in plant systems biology. Kusano, M.[1,2]. RIKEN Plant Science Center[1], Kihara Institute for Biological Research, Yokohama City University[2].

Metabolomics is one of the "omics" technologies and the goal of metabolomics is to quantify all the metabolites (metabolome) in the cell. We have developed the multiple mass spectrometry (MS) based metabolomics pipeline. We applied metabolomics to understand hidden metabolic networks in Arabidopsis (1). We chose two representative mutants, methionine-over accumulation 1 (mto1) and transparent testa4 (tt4) for primary and secondary metabolism. We captured genotype-dependent changes of metabolite levels and metabolite fluctuations in individual samples of wild-type and the mutants by using chromatography time-of-flight mass spectrometry (GC-TOF-MS). In metabolic networks, mto1 lost their metabolic stability, while tt4 enhanced a metabolic network of a backup pathway to compensate the lost physiological functions even under the normal growth condition. We also investigated the extent of methionine over-accumulation toward a whole metabolism in Arabidopsis using three different mto mutants (mto1, mto2 and mto3) (2). As an extended research for tt4, we treated tt4, tt5 and sinapoylglucose accumulator 1 (sng1) by supplementary UV-B light. These mutants cannot produce UV-B absorbing metabolites such as flavonoids (for tt4 and tt5) and sinapoylmalate (for sng1).Samples were analyzed by GC-TOF-MS and liquid chromatography - quadrupole-TOF-MS to know global regulation of metabolism in response to the stress (3).Since metabolomics requires no genome information, we can apply metabolomics to any organisms. Application examples of metabolomics for crops and vegetables will be also presented.

(1) BMC Syst Biol 1. 53 (2007).
(2) Amino Acids 39. 1013-1021 (2010).
(3) Plant J 67. 354-369 (2011).

Nitrogen regulatory networks controlling plant root growth. Gutiérrez, R.[1]. FONDAP Center for Genome Regulation, Millennium Nucleus Center for Plant Functional Genomics, Departamento de Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile, Alameda 340, Santiago, Chile[1].

Nitrogen (N)-based fertilizers increase agricultural productivity but have detrimental effects on the environment and human health. Research is generating improved understanding of the signaling components plants use to sense N and regulate metabolism, physiology, and growth and development. However, we still need to integrate these regulatory factors into signal transduction pathways and connect them to downstream response pathways. We used systems approaches to identify gene regulatory networks involved in N responses using Arabidopsis thaliana as a plant model system. Using next generation sequencing, microarray technologies and integrative network bioinformatics tools we are dissecting nitrate-regulatory networks controlling root growth. We will discuss our current experimental efforts towards mapping gene networks leading to nitrate induced changes in root system architecture. We will also discuss our new bioinformatics tools to identify new components of the nitrogen response in *Arabidopsis thaliana*. Systems biology approaches is accelerating the identification of new components and N-regulatory networks linked to other plant processes. A holistic view of plant N nutrition should open avenues to translate this knowledge into effective strategies to improve N-use efficiency and enhance crop production systems for more sustainable agricultural practices.

Novel dimensions to epigenetic regulation in biological control and cancer. Stein, G.S.[1], Lian, J.B.[1], Stein, J.L.[1], van Wijnen, A.J.[1]. Department of Cell Biology, University of Massachusetts Medical School, Worcester, Massachusetts[1].
Modifications, particularly subtle changes, in control of gene expression are pivotal for biological control and decisive prognostic indicators of transformation and tumorigenesis. Physiological regulation as well as the onset and progression of cancer are functionally linked with aberrant genetic (DNA-encoded) and epigenetic (non-DNA-encoded) mechanisms that influence the transmission and retention of regulatory information during mitotic division. The rapid and persistent responsiveness of epigenetic control provides an important dimension for assaying the fidelity of biologic regulation and perturbations that are acquired in the initial stages of cancer.
The significance of epigenetic control from a regulatory perspective is emphasized by the capacity to convey regulatory information from parental to progeny cells during mitotic division through non-DNA-encoded mechanisms that include DNA and histone modifications, microRNAs, and mitotic retention of transcription factors at chromosomal loci of target genes. The composite parameters of epigenetic control provide informative signatures that can support stringent risk assessment for compromised gene expression that accompanies tumorigenesis.

Developing genetically engineered vectors to treat human gastric tumors. Montecino, M.[1]. Center for Biomedical Research and FONDAP Center for Genome Regulation, Faculty of Biological Sciences and Faculty of Medicine, Universidad Andres Bello, Santiago, Chile[1].
Although there has been considerable improvement in the surgical techniques to treat gastric cancer, the clinical response of advanced gastric cancer patients is generally poor, with survival rates in the range between 5 and 15%. In this scenario, development of alternative strategies such as gene therapy, become relevant to treat this illness in the future. Here, we demonstrate that a segment of the human REG1A gene promoter extending from -571 to +75 relative to the transcription start site is sufficient to selectively drive the expression of a heterologous gene in human gastric tumor cells. We also report the generation of a REG1A-based genetically-engineered vector (pART) that exhibits further increased promoter activity in these tumor-derived gastric cells. In addition to the hREG1A promoter sequence (REG1A-571), pART includes nucleosome-positioning and nucleosome-excluding sequences previously identified at the rat osteocalcin gene promoter. This DNA segment operatively connects REG1A-571 with a sequence of tandem-repeats of Wnt signaling-responsive elements (TBE), recently identified by our research team *at the human COX-2 gene promoter. Our results support the future* use of pART as a basic construct for efficient and cell-specific gene delivery to gastric tumors in humans.FONDAP 15090007.

Rheb-to-AMPK in cancer: unlikely to have good intentions. Lacher, M.[1], Campos, T.[2], Armijo, M.[2], Ziehe, J.[1], Zhu, Z.[1], Pincheira, R.[2], **Castro, A.**[2]. Helen Diller Family Comprehensive Cancer Center, University of California San Francisco, CA, USA[1], Departamento de Bioquímica y Biología Molecular, Universidad de Concepción, Chile[2].
Mammalian target of rapamycin complex 1 (mTORC1) kinase stimulates cell growth by integrating environmental signals for cell division with availability of adequate energy and nutrients. According with its cellular function, mTORC1 is found hyperactivated in several genetic disorders and cancer. Thus, specific mTORC1 inhibitors are in clinical trials for different types of cancer. While the therapeutic efficacy of these inhibitors is not yet proven, a better understanding of how mTORC1 signaling is regulated could provide additional, better or alternative targets for cancer treatment. mTORC1 activity is repressed by a complex of two tumor suppressor proteins, TSC1 and TSC2 (TSC1/2). We previously demonstrated that TSC2 functions as a GTPase-activating protein (GAP) for the inactivation of a small GTPase known as Rheb and thereby mTORC1, indicating that Rheb is the molecular link between TSC1/2 and mTORC1. Although there is strong evidence demonstrating that the Rheb/mTORC1 signaling promotes tumorigenesis, we recently found that aberrant Rheb activation could also contribute to disease independently of its ability to activate mTORC1 by regulating the activities of adenosine 5'-monophosphate-activated protein kinase (AMPK) and of the cyclin-dependent kinase inhibitor $p27^{KIP1}$. Rheb-induced activation of AMPK is surprising and in principle incompatible with Rheb's oncogenic activity. In fact, as AMPK inhibits energy-consuming anabolic mechanisms supporting cell growth, AMPK plays tumor suppressive roles. However, there are evidences for ambivalent roles for AMPK in tumorigenesis. The implications of a Rheb-to-AMPK signaling for disease progression and treatments for cancer will be discussed. USA.DOD(TS043006)/DIUC(210.037.011-1.0)/ FONDECYT(1120923).

Tumor suppression and metastasis in melanomas and gastric cancer. Quest, A.F.[1]. Centro de Estudios Moleculares de la Célula (CEMC), Facultad de Medicina, Universidad de Chile[1].
The discussion here will focus on how some proteins alter their function in a context-dependent fashion in cancer using melanomas and gastric cancer cells as experimental models. In melanoma development, loss of E-cadherin and upregulation of N-cadherin are associated with transition to a metastatic phenotype. The role of caveolin-1 (Cav-1) in this context is not known, however meta-analysis of available expression data in melanoma lines suggests that Cav-1 is upregulated in this transition. In a pre-clinical C57BL6 mouse model, expression of Cav-1 alone in B16F10 mouse melanomas reduced subcutaneous tumor growth but dramatically increased metastasis to the lung when injected intravenously. Intriguingly, co-expression of E-cadherin with Cav-1 completely suppressed metastasis to the lung, but cooperated with Caveolin-1 in tumor suppression. Thus E-cadherin functions as a "molecular switch" that promotes the protective role of Cav-1 in melanomas in vivo. Chronic exposure to H. pylori promotes cell death and the onset of gastric atrophy, early events associated with the genesis of gastric cancer. In human gastric tissue samples and cell lines, H. pylori infection is linked to loss of survivin and increased cell death. More recent *results indicate that survivin* downregulation is attributable to a post-translational mechanism involving proteasome-mediated degradation of the protein in a manner dependent on bacterial gamma glutamyl transpeptidase (GGT) activity. Therefore, contrary to general thinking, survivin in the context of the gastric epithelium represents a factor that protects against H. pylori -induced disease. Acknowledgements: FONDECYT-FONDAP15010006, FONDECYT1090071, ACT1111 (AFGQ).

Beneficial effects of L-carnitine in arterial hypertension and hypertension-related organ damage. Mate, A.[1], Zambrano, S.[1], Blanca, A.[1], Ruiz-Armenta, M.V.[1], Miguel-Carrasco, J.L.[1], Salsoso, R.[1], Guzmán-Gutiérrez, E.[2], Pardo, F.[2], Leiva, A.[2], Sobrevia, L.[2], **Vázquez, C.M.**[1]. Department of Physiology, Faculty of Pharmacy, Universidad de Sevilla, Spain[1], Cellular and Molecular Physiology Laboratory (CMPL), Division of Obstetrics and Gynecology, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile, Chile[2].

L-carnitine (LC) is a natural compound whose main physiological function is the transport of fatty acids inside the mitochondria for ATP production. Exogenous administration of LC is mainly used in those pathologies presenting with a deficit of this substance; however, evidences suggest that a supplementation with LC may be relevant in the pathophysiology of different diseases, including arterial hypertension. Arterial hypertension is one of the most important diseases worldwide and it is a main risk factor for the development of cardiovascular and renal diseases. It has been demonstrated that oxidative stress, inflammation and fibrotic processes are involved in molecular mechanisms contributing to the pathophysiology of hypertension and all hypertension-related organ damage. With this background we will try to demonstrate the beneficial effect of LC in arterial hypertension. The hypotensive, vasodilator, antioxidant, anti-inflammatory and antifibrotic properties of LC has been proved in several rat models of arterial hypertension, such as SHR, Spontaneously Hypertensive rats, and rats treated with NG-nitro-L-arginine methyl ester (L-NAME). All these experiments suggest that LC may be considered as a good candidate for treatment of arterial hypertension and hypertension-related organ diseases. Supported by Instituto de Salud Carlos III, Subdirección General de Evaluación y Fomento de la Investigación, PN de I+D+I 2008-2011 (PS09/01395) and AECID A1/036123/11 (Spain). CONICYT (ACT-73 PIA, AT-24120944), FONDECYT (1110977, 11110059, 3130583), and CONICYT-PhD fellowship (EG-G), Chile.

Maternal supraphysiological hypercholesterolemia in pregnancy leads to placental endothelial dysfunction. **Leiva, A.**[1], Diez de Medina, C.[1], Guzmán-Gutiérrez , E.[1], Abarzúa, F.[2], Pardo, F.[1], Sovrevia, L.[1]. Cellular and Molecular Physiology Laboratory (CMPL), Division of Obstetrics and Gynecology, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile[1], Clínica Alemana, Temuco, Chile[2].

Maternal physiological hypercholesterolemia (MPH) occurs in pregnancy assuring fetal growth and development but maternal supraphysiological hypercholesterolemia (MSPH) leads to aortic atherosclerosis in the fetus and children. Since endothelial function is altered by hypercholesterolemia we hypothesize that MSPH alters L-arginine/NO pathway in human umbilical vein endothelial cells (HUVEC) leading to altered vascular reactivity. MSPH (maternal total cholesterol >280 mg/dL at term) was associated with reduced endothelium-dependent (calcitonin gene-related peptide) vasodilation compared with MPH (IC50 0.50 - 0.003 v/s 0.15 - 0.002 nM); however, SNP-vasodilatation (endothelium-independent) was unaltered in MSPH. NOS activity (L-[3H]citrulline formation from L-[3H]arginine) was lower in MSPH (29%) compared with MPH without changes in eNOS expression. L-Arginine transport (L-[3H]arginine 30-500 ?M, 1 minute) maximal velocity was higher in MSPH compared with MPH (Vmax 12 - 3 v/s 5 – 1 pmol/Mμ g protein/minute) without significant changes in apparent Km or human cationic amino acid transporter 1 protein expression. Arginase II activity (urea formation from L-arginine) and expression was increased in MSPH cells (1.3 - 0.08 and 2.4 - 0.64 folds compared with MPH, respectively). In umbilical veins from MSPH the intimal-medial ratio (hematoxyline-eosin and Verhoeff stain) was higher compared with MSPH. We propose that MSPH associates with altered L-arginine bioavailability for NOS in HUVEC leading to reduce umbilical vein reactivity and changes in its structural conformation, a likely key phenomenon in MSPH-associated adult cardiovascular disease. Support: CONICYT (ACT-73 PIA, AT-24120944), FONDECYT (11110059, 1110977, 3130583), and CONICYT-PhD fellowship (EG-G), Chile.

In utero programming of cardiovascular disease by maternal hypercholesterolemia. **Palinski, W.**[1]. Department of Medicine, University of California, San Diego, USA[1].

Substantial epidemiological evidence links conditions in utero with adult cardiovascular disease (CVD). The increasing prevalence of maternal obesity and dysmetabolic conditions in developed countries is therefore expected to increase CVD prevalence in offspring, but the causes of in utero programming and the mechanisms by which it promotes atherosclerosis and diabetes remain largely unknown. The most important (and least explored) translational aspect of in utero programming is the possibility of achieving life-long benefits for offspring by brief interventions in mothers prior to or during pregnancy. We previously identified maternal hypercholesterolemia and the ensuing oxidative stress as a major cause of atherogenic programming in humans and experimental models, and showed that lipid-lowering or antioxidant interventions during pregnancy reduce atherosclerosis in offspring. Some of the mechanisms by which in utero programming may affect CVD were identified, including systemic effects on inflammation. Maternal adaptive immunity to oxidative neoepitopes programs beneficial lymphocyte-dependent IgM responses in offspring. Maternal immunomodulation may thus be a way to prevent pathogenic programming, e.g. by reducing fetal exposure to oxidative stress and by inducing protective immune programming. In experimental models maternal immunization with oxidized lipoproteins reduces the susceptibility to atherosclerosis in offspring, and protects them against insulin resistance and type 2 diabetes. The possibility of both promoting and inhibiting offspring diseases should also help to identify relevant mechanisms and targets for intervention, as well as early markers of in utero programming. FONDECYT (11110059, 1110977) and CONICYT (ACT-73 PIA)-Chile international collaboration support.

The role of the proline-rich tyrosine kinase PYK2 and tyrosine phosphorylation in the regulation of eNOS. **Fleming, I.**[1]. Institute for Vascular Signaling, Centre for Molecular Medicine, Goethe University, Frankfurt am Main, Germany.[1].

The proline-rich tyrosine kinase (PYK2) is a focal adhesion-related tyrosine kinase that can be activated by oxidative stress. Given that the endothelial nitric oxide synthase (eNOS) plays an important role in the endothelial function we studied the effects of PYK2 activation/ inhibition on eNOS signalling. In endothelial cells, angiotensin II (AngII) enhanced the tyrosine phosphorylation of eNOS in an AT1, H2O2 and PYK2-dependent manner. Low concentrations (1-100 Mμ mol/L) of H2O2 stimulated the phosphorylation of eNOS Tyr657 without affecting that of Ser1177, and attenuated basal and agonist-induced NO production. In isolated murine aortae, H2O2 (30 Mμ mol/L) induced phosphorylation of eNOS on Tyr657 and impaired acetylcholine-induced relaxation. Endothelial overexpression of a dominant negative PYK2 mutant protected against H2O2-induced endothelial dysfunction. Correspondingly, carotid arteries from eNOS-/- mice overexpressing a eNOS Y657F mutant were also protected against H2O2. In vivo three weeks treatment of wild-type, but not Nox2y/- mice, with AngII considerably increased levels of Tyr657 phosphorylated eNOS in aortae and this was associated with a clear impairment in endothelium-dependent vasodilatation in the wild-type, but not the Nox2y/- mice. Thus, endothelial PYK2 activation by AngII and H2O2 elicits the phosphorylation of eNOS on Tyr657, attenuating NO production and endothelium-dependent vasodilatation. This mechanism may contribute to the endothelial dysfunction observed in cardiovascular diseases associated with increased activity of the renin-angiotensin system and elevated redox stress. FONDECYT (1110977, 11110059) and CONICYT (ACT-73 PIA)-Chile international collaboration support.

NEW MEMBERS SESSIONS

Energy-preserving effects of IGF-1 antagonize starvation-induced cardiac autophagy. Troncoso, R.[1], Hill, J.A.[2], Abel, E.D.[3], LeRoith, D.[4], Lavandero, S.[1,2,5]. Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/Faculty of Medicine, University of Chile[1], University of Texas Southwestern Medical Center, Dallas, USA[2], University of Utah School of Medicine, Salt Lake City, USA[3], The Mount Sinai School of Medicine, New York, USA[4], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile[5].

IGF-1 is known to exert cardioprotective actions. However, it remains unknown if autophagy, a major adaptive response to nutritional stress, contributes to IGF-1-mediated cardioprotection. We subjected cultured rat cardiomyocytes, as well as live mice, to nutritional stress and assessed cell death and autophagic rates. Nutritional stress induced by serum/glucose deprivation strongly induced autophagy and cell death, and both responses were inhibited by IGF-1. The Akt/mTOR pathway mediated the effects of IGF-1 upon autophagy. Importantly, starvation also decreased intracellular ATP levels and oxygen consumption leading to AMPK activation; IGF-1 increased mitocondrial Ca^{2+} uptake and mitochondrial respiration in nutrient-starved cells. IGF-1 also rescued ATP levels, reduced AMPK phosphorylation and increased p70S6K phosphorylation, which indicates that in addition to Akt/mTOR, IGF-1 inhibits autophagy by the AMPK/mTOR axis. In mice harbouring a liver-specific *igf1* deletion, which dramatically reduces IGF-1 plasma levels, AMPK activity and autophagy were increased, and significant heart weight loss was observed in comparison with wild-type starved animals, revealing the importance of IGF-1 in maintaining cardiac adaptability to nutritional insults *in vivo*. Our data support the cardioprotective actions of IGF-1, which, by rescuing the mitochondrial metabolism and the energetic state of cells, reduces cell death and controls the potentially harmful autophagic response to nutritional challenges. IGF-1, therefore, may prove beneficial to mitigate damage induced by excessive nutrient-related stress, including ischemic disease in multiple tissues. Supported by Anillo ACT1111 (SL).

HMGB proteins modify the activity of ATP-dependent chromatin remodeling complexes and its association to gene promoters. Hepp, M.I.[1], Smolle, M.[2], Alarcón, V.[1], Workman, J.L.[2], Gutiérrez, J.[1]. Departamento de Bioquimica y Biologia Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile.1, Stowers Institute for Medical Research, Kansas City, Missouri, USA.[2].

Chromatin dynamics governs the access of DNA-binding proteins to the DNA. Among the proteins and protein complexes affecting this dynamics are ATP-dependent chromatin remodeling machines, being the SWI/SNF complex its founding member. This complex is involved in transcriptional regulation of a large number of genes. Playing also a significant role in chromatin dynamics are HMGB proteins. It has been shown that HMGB1 stimulates ATP-dependent remodeling activity, although little is known about the affect of HMGB proteins on SWI/SNF activity and there are no studies addressing whether these proteins affect the physical presence of SWI/SNF on gene regulatory regions.In order to analyze how yeast HMGB proteins influence the activity of yeast SWI/SNF, we performed in vitro nucleosome remodeling assays, using three yeast HMGB type proteins and human HMGB1. To study the influence of yHMGB proteins on the physical presence of SWI/SNF on gene regulatory regions we performed ChIP-chip assays for Snf5 (a SWI/SNF subunit) using different yeast strains, including deletion mutants of the HMGB proteins under study. We also studied the genome-wide relative enrichment of these HMGB proteins using TAP-tag strains. Our results indicate that yeast HMGB proteins enhance the activity of remodeling complexes in vitro. Our ChIP-chip analyses indicate that yeast HMGB proteins co-localize with SWI/SNF on promoter regions of several genes. Importantly, deletion mutants show a reduction in the physical presence of SWI/SNF on promoter regions of a significant number of these genes.Fondecyt-1085092; DIUC-211.037.014-1.0; AT-24100076; P-75110059.

The first transmembrane domain (TM1) of beta2-subunit binds to the transmembrane domain S1 of alpha-subunit in BK potassium channels. Morera, F.J.[1], Alioua, A.[2], Kundu, P.[2], Salazar, M.[3], González, C.[3], Martinez, A.D.[3], Stefani, E.[2], Toro, L.[2], Latorre, R.[3]. Institute of Pharmacology and Morphophysiology, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Valdivia, Chile[1], Division of Molecular Medicine, Department of Anesthesiology, University of California, Los Angeles, USA[2], Centro Interdisciplinario de Neurociencia de Valparaiso, Chile[3].

The BK channel is one of the most broadly expressed ion channels in mammals. In many tissues, the BK channel pore-forming alpha-subunit is associated to an auxiliary beta-subunit that modulates the voltage- and Ca2+-dependent activation of the channel. Structural components present in beta-subunits that are important for the physical association with the alpha-subunit are yet unknown. Here, we show through co-immunoprecipitation that the intracellular C-terminus, the second transmembrane domain (TM2) and the extracellular loop of the beta2-subunit are dispensable for association with the alpha-subunit pointing transmembrane domain 1 (TM1) as responsible for the interaction. Indeed, the TOXCAT assay for transmembrane protein?protein interactions demonstrated for the first time that TM1 of the beta2-subunit physically binds to the transmembrane S1 domain of the alpha-subunit. Acknowledgments: Dirección de Investigación y Desarrollo (DID) de la Universidad Austral de Chile. This work was supported by FONDECYT Grants 1110430 (to R.L.), 1090573 (to A.D.M.) and 1120802 (to C.G.) DID-UACH Grant S-2012-13 (to F.J.M.),

Paleoenzymology of glucokinase/phosphofructokinase activity in the ADP-dependent sugar kinases family by resurrection of ancestral enzymes. Castro-Fernandez, V.[1], Herrera-Morandé, A.[1], Guixé, V.[1]. Laboratorio de Bioquímica y Biología Molecular, Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile.[1].

Promiscuous enzyme activities have been proposed as starting points for the generation of new specificities through evolution, therefore, commonly non-specific enzymes are proposed as ancestral proteins. The ADP-dependent sugar kinases family has two specificities; phosphofructokinase (PFK) and glucokinase (GK), being most of the experimentally characterized enzymes highly specific for glucose or fructose-6P. Interestingly, the enzyme from *Methanocaldococcus jannaschii* has been described as bi-functional and capable to phosphorylate both sugars. For this reason, it was proposed to be an ancestral form for the family. In this work we reconstructed the phylogeny of this family and the evolutionary history of the use of substrates through the estimation of ancestral states of discrete traits based on likelihood. We inferred, synthesized and expressed the gene for the last common ancestor of phosphofructokinases from the *Thermococcales* and *Methanococcales* groups (ancMT) and the PFK ancestor of the *Thermococcales* group (ancT) and then, we compared the substrate specificity of them with the estimation of ancestral states of discrete traits. The results indicate that the enzymes from *Methanococcales* are not specific phosphofructokinases as are believed to date. These enzymes are able to phosphorylated both glucose and fructose-6P whereas only the enzymes from the *Thermococcales* group are specific for fructose-6P. We built homology models of the ancestral proteins structures and use them to performed ligand-docking experiments and molecular dynamics simulations, which allow us to identify key residues for fructose-6P specialization. Fondecyt-1110137. Beca CONICYT Doctorado.

Characterization of a putative grapevine Zn transporter, VvZIP3, suggests its involvement in early reproductive development in *Vitis vinifera* **L. Gainza-Cortés, F.**[1,2], Pérez-Diaz , R.[2], Pérez-Castro, R.2,3, Tapia, J.2, Casaretto, J.A.2, González, S.2, Ruiz-Lara, S.2, González, E.2. Centro de Estudios Avanzados en Fruticultura[1], Universidad de Talca[2], Universidad Catolica del Maule[3].

Zinc (Zn) deficiency is one of the most widespread mineral nutritional problems that affect normal development in plants. Because Zn cannot passively diffuse across cell membranes, it must be transported into intracellular compartments for all biological processes where Zn is required. Several members of the ZIP gene family have been characterized in plants, and have shown to be involved in metal uptake and transport. We identified and characterized a putative Zn transporter from berries of Vitis vinifera, named VvZIP3. *VvZIP3* is mainly expressed in reproductive tissue - specifically in developing flowers - which correlates with the high Zn accumulation in these organs. Contrary to this, the low expression of *VvZIP3* in parthenocarpic berries shows a relationship with the lower Zn accumulation in this tissue than in normal seeded berries. The predicted protein sequence indicates strong homology with several members of the ZIP family from Arabidopsis thaliana and other species. Moreover, VvZIP3 complemented the growth defect of a yeast Zn-uptake mutant, ZHY3, and is localized in the plasma membrane of plant cells, suggesting that VvZIP3 has the function of a Zn uptake transporter. Our results suggest that VvZIP3 might play a role in Zn uptake and distribution during the reproductive development in Vitis vinifera and indicate that the availability of this micronutrient may be relevant for normal berry development. CEAF/ CONICYT-REGIONAL R08I1001, FONDECYT 1120871.

Endocytic trafficking towards the vacuole plays a key role in the auxin receptor SCF^{TIR}-independent mechanism of lateral root formation in *A. thaliana.* **Pérez-Henríquez, P.**[1,2], Raikhel, N.V.[3], Norambuena, L.[1,2]. Plant Molecular Biology Laboratory, Department of Biology, Faculty of Sciences, University of Chile, Santiago, Chile[1], Millenium Nucleus in Plant Cell Biotechnology, Chile[2], Center for Plant Cell Biology and Department of Botany and Plant Sciences, University of California, Riverside, CA 92521, USA[3].

Plant developmental plasticity plays a pivotal role in responding to environmental conditions. One of the most plastic plant organs is the root system. Different environmental stimuli such as nutrients and water deficiency may induce lateral root formation to compensate for a low level of water and/or nutrients. It has been shown that the hormone auxin tunes lateral root development and components for its signaling pathway have been identified. Using chemical biology, we discovered an *Arabidopsis thaliana* lateral root formation mechanism that is independent of the auxin receptor SCF^{TIR}. The bioactive compound Sortin2 increased lateral root occurrence by acting upstream from the morphological marker of lateral root primordium formation, the mitotic activity. The compound did not display auxin activity. At the cellular level, Sortin2 accelerated endosomal trafficking, resulting in increased trafficking of plasma membrane recycling proteins to the vacuole. Sortin2 affected Late endosome/PVC/MVB trafficking and morphology. Combining Sortin2 with well-known drugs showed that endocytic trafficking of Late endosome/PVC/MVB towards the vacuole is pivotal for Sortin2-induced SCF^{TIR}-independent lateral root initiation. Our results revealed a distinctive role for endosomal trafficking in the promotion of lateral root formation via a process that does not rely on the auxin receptor complex SCF^{TIR}. This work was supported by Fondo Nacional de Desarrollo Tecnológico FONDECYT (11080240 to P.P-H. and L.N.); Plant Cell Biotechnology-Millenium Nucleus (P006-065-F to P.P-H. and L.N.), and National Science Foundation (MCB0515963 to N.V.R.).

IRE1/bZIP60-mediated unfolded protein response plays distinct roles in plant immunity and abiotic stress responses. **Moreno, A.A.**[1], Blanco, F.[1], Moreno, I.[1], Orellana, A.[1]. FONDAP Centro de Regulación del Genoma, Núcleo Milenio en Genómica Funcional de Plantas, Centro de Biotecnología Vegetal, Facultad de Ciencias Biológicas, Universidad Andrés Bello.[1]. Sponsored by Leon, G.

Endoplasmic reticulum (ER)-mediated protein secretion and quality control have been shown to play an important role in immune responses in both animals and plants. In mammals, the ER membrane-located IRE1 kinase/endoribonuclease, a key regulator of unfolded protein response (UPR), is required for massive secretion of immunoglobulins. In counterpart, plant cells can secrete the so-called pathogenesis-related (PR) proteins upon pathogen challenge. However, whether IRE1 plays any role in plant immunity is not known. *Arabidopsis thaliana* has two copies of IRE1, IRE1a and IRE1b. Here, we show that both IRE1a and IRE1b are transcriptionally induced during ER stress. In addition, we found that IRE1a plays a predominant role in the secretion of PR proteins upon SA treatment. Consequently, the ire1a mutant plants show enhanced susceptibility to a bacterial pathogen and are deficient in establishing systemic acquired resistance (SAR). We further demonstrate that the immune deficiency in ire1a is due to a defect in IRE1-mediated cytoplasmic splicing of the bZIP60 mRNA, which encodes a transcription factor involved in the UPR. Consistently, IRE1a is preferentially required for bZIP60 splicing upon pathogen infection, while IRE1b plays a major role upon Tunicamycin (Tm)-induced stress. We also show that SA-dependent induction of UPR-responsive genes is altered in the bzip60 mutant. These results indicate that the IRE1/bZIP60 branch of UPR is a part of the plant response to pathogens and that IRE1 has both bZIP60-dependent and bZIP60-independent functions in plant immunity. *AAM is recipient of CONICYT doctoral scholarship.

The Arabidopsis JAZ2 promoter contains a G-box and thymidine-rich module that are necessary and sufficient for jasmonate-dependent activation by MYC transcription factors and repression by *JAZ proteins.* ***Figueroa, P.***[1], *Browse, J.*[2]. Escuela de Biotecnología, Facultad de Ciencias, Universidad Santo Tomás, Santiago, Chile[1], Washington State University, Pullman, Washington 99164-6340, USA[2].

Jasmonate (JA) is a critical hormone for both plant defense and reproductive development. Until now, early JA-responsive promoters have not been characterized. To identify the cis-acting DNA element involved in the early JA response at the transcriptional level, we analyzed the promoter of the Arabidopsis gene encoding jasmonate-ZIM domain2 protein (JAZ2). The full-length *JAZ2* promoter in *JAZ2::GUS* plants is active in vegetative and reproductive tissue, with expression in stamen filaments being dependent on JA-biosynthesis. We identified a G-box element in the *JAZ2* promoter that is required for JA-mediated promoter activation in carrot protoplasts and Arabidopsis seedlings. Three copies of a G?box and flanking sequences has autonomous JA-dependent activity and was transactivated by MYC2, MYC3 and MYC4 transcription factors in carrot protoplasts. Expression of MYC2, MYC3 or MYC4 fused to an EAR (ETHYLENE RESPONSE FACTOR-associated amphiphilic repression) motif, or expression of JAZ1 or JAZ6 all repressed JA- and MYC-dependent activation of the JAZ2 promoter. A thymidine-rich sequence 3' to the G-box was required for full JA activation of the *JAZ2* promoter. Because the G-box is also present in genes unresponsive to JA, we propose that this thymidine-rich sequence together with the G-box provides JA specificity to promoters induced early in the JA response. Together, these results indicate that an extended G-box located in the promoter region of an early JA-responsive gene is required for gene induction in vivo, probably through selective binding to MYC2, MYC3 and MYC4 transcription factors.

Glycosylations in the globular head of the hemagglutinin protein modulate the virulence and antigenic properties of the H1N1 influenza viruses. Medina, R.A.[1,2,3], Stertz, S.[2,3,4], Manicassamy, B.[2,3], Sun, X.[5], Albrecht, R.A.[2,3], Belshe, R.B.[6], Frey, S.E.[6], Tumpey, T.M.[5], García-Sastre, A.[2,3,7]. Laboratory of Molecular Virology, Instituto Milenio en Inmunología e Inmunoterapia, Centro de Investigaciones Médicas y División de Pediatría, Facultad de Medicina, Pontificia Universidad Católica de Chile[1], Department of Microbiology[2], Global Health and Emerging Pathogens Institute, Mount Sinai School of Medicine, New York, USA[3], Institute of Medical Virology, University of Zurich, Zurich, Switzerland[4], Influenza Division, National Center for Immunization and Respiratory Disease, Centers for Disease Control and Prevention, Atlanta, USA[5], Saint Louis University School of Medicine, Saint Louis University, St. Louis, USA[6], Department of Medicine, Division of Infectious Diseases, Mount Sinai School of Medicine, New York, USA[7]. Sponsored by López-Lastra, M.

The seasonal circulation of the 2009 pandemic H1N1 (pH1N1) influenza virus in humans will likely induce its antigenic drift. Over time, seasonal strains have acquired glycosylation sites in the globular head of their hemagglutinin (HA), which can shield antigenically relevant regions. We engineered recombinant (rpH1N1) viruses containing additional HA glycosylations reflecting their temporal appearance in previous seasonal H1N1 viruses. Additional glycosylations resulted in attenuation in mice and ferrets, while deleting HA glycosylations from a contemporary seasonal H1N1 strain increased its pathogenicity in mice. Sera from mice infected with wild type (WT) rpH1N1 virus had a reduced HA inhibitory (HI) activity against rpH1N1viruses glycosylated at sites 144 or 144-172, indicating that the polyclonal antibody response elicited by WT pH1N1 HA seems to be against an immunodominant region, likely site Sa, shielded by glycosylation 144. Sera from humans vaccinated with inactivated pH1N1 also showed reduced activity against the 144 and 144-172 mutant viruses. Remarkably, sera from mice infected with a virus glycosylated at position 144 showed a broader polyclonal response that cross-neutralized all WT and glycosylation mutant viruses. Mice infected with a recent seasonal virus in which glycosylation sites 71, 142 and 177 were removed, elicited antibodies that protected against challenge with the antigenically distant pH1N1 virus. Thus, acquisition of HA glycosylations in human H1N1 viruses affects their pathogenicity, ability to escape from polyclonal antibodies elicited by previous influenza virus strains; and can also induce cross-reactive antibodies against antigenic variants.CONICYT 79100014.

Herp depletion protects from protein aggregation by up-regulation of Atg5 and Beclin-1 during early stress conditions. Quiroga, C.[1], Gatica, D.[1], Paredes, F.[1], Bravo, R.[1], Troncoso, R.[1], Pedrozo, Z.[1,2], Rodríguez, A.E.[1], Vicencio, J.M.[3], Chiong, M.[1], Hetz, C.[1,2], Lavandero, S.[1,2,4]. Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/Faculty of Medicine, Universidad de Chile[1], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile[2], Department of Cell and Developmental Biology and Consortium for Mitochondrial Research, University College London, United Kingdom[3], Department of Internal Medicine, University of Texas Southwestern Medical Center, USA[4].

Herp, an endoplasmic reticulum (ER) membrane protein activated during the unfolded protein response (UPR), mediates ER stress adaptation and promotes ER-associated protein degradation (ERAD) pathway. However, Herp contribution to autophagy and its connection to other types of stress are unknown. Here we report that Herp negatively regulates the clearance of poly-ubiquitin (poly-Ub) protein aggregates. Accumulation of poly-Ub protein aggregates induced by proteasome inhibition or nutritional stress induced by amino acid and glucose deprivation was reduced in stable Herp knockdown cells (shHerp). Cell death induced by glucose deprivation, but not amino acid deprivation or MG132, was inhibited by enhancing poly-Ub aggregates removal. Under glucose deprivation increased both LC3-II levels and GFP-LC3 puncta were observed in shHerp cells compared to controls, which was associated to Beclin-1 and Atg5 up-regulation. These results showed that protein aggregate clearance by an enhanced autophagic flux is necessary for survival. Our results suggest that an early autophagic Herp-dependent reduction of poly-Ub aggregates is a stress defense mechanism. This response effectively protects from acute stress such as glucose deprivation, but not from chronic stress conditions such as ER stress, amino acid deprivation or protein aggregation. Supported by Anillo ACT1111 (MC and SL).

Insulin-dependent GLUT4 translocation requires concerted NADPH oxidase-RyR1 axis and IP3R activation in skeletal muscle cells. Contreras-Ferrat, A.[1], Klip, A.[2], Lavandero, S.[1,3], Jaimovich, E.[1]. Center for Molecular Studies of the Cell and Biomedical Sciences Institute, Faculty of Medicine, Universidad de Chile[1], Cell Biology Program, The Hospital for Sick Children, Toronto, Ontario, Canada M5G [2], Department of Internal Medicine (Cardiology), University of Texas Southwestern Medical Center, Dallas, Texas 75235, USA[3].

In insulin-regulated GLUT4 traffic the roles of Ca^{2+} and ROS signaling have been poorly understood. L6GLUT4*myc* myotubes exposure to low concentration of H_2O_2 increase GLUT4*myc* translocation to cell surface and was additive to insulin co-stimulation. Contrary, high concentration of H_2O_2 abolishes insulin effect. Pharmacological and molecular antioxidants agents reduced GLUT4*myc* externalization, as did both NADPH oxidase inhibitor apocynin and p47phox knockdown. Insulin-induced an increase in S-glutathionylation state of RyR1, detected by ligand proximity assay, while activation and inhibition of RyR1 increase and decrease insulin effect respectively. Insulin increase H_2O_2 production (cytosolic HyPer) and intracellular Ca^{2+} levels (FLUO4-AM), both inhibited by N-acetylcysteine. The increase of glucose uptake and GLUT4*myc* translocation induced by insulin were independent of extracellular Ca^{2+} and blocked by intracellular Ca^{2+} chelator BAPTA-AM or cytosol-directed parvalbumin expression. Ryanodine or xestospongin reduced GLUT4*myc* exposure with additive effect. Ionomycin and 4-Chloro-m-Cresol increase GLUT4*myc* translocation and were insulin additive. Myotubes maintained in Ca^{2+}-free media and pre-incubated with ionomycin, 4-CMC or thapsigargin, insulin did not raise GLUT4*myc* levels to surface. Insulin increases IP_3 production that was completely inhibited by U73122 pre-incubation. Both, PI3Kgamma kinase dead construct and PLC inhibitor U73122 reduce insulin-dependent GLUT4*myc* translocation to cell surface. These data suggest that insulin increase NADPH oxidase-dependent H_2O_2 production loading RyR1 activation by ROS modification and induces IP_3 production via PI3Kgamma/PLC activation. Both Ca^{2+} channels are cooperatively involved to foster GLUT4 translocation induced by insulin. ACT1111, FONDECYT 3110170, 1110467.

Muscle glycogen synthase isoform is responsible for testicular glycogen synthesis: glycogen overproduction induces apoptosis in male germ cells. Villarroel-Espindola, F.[1], Mancilla, H.[1], Vander Stelt, K.[1], Maldonado, R.[1], Acuña, A.[1], Covarrubias, A.[1], López, C.[1], Angulo, C.[2], Castro, M.[1], Slebe, J.C.[1], Durán, J.[3], Rocha-García, M.[3], Guinovart, J.J.[3], Concha, I.I.[1]. Instituto de Bioquímica y Microbiología, Universidad Austral de Chile, Valdivia, Chile[1], Instituto de Química, Universidad Austral de Chile, Valdivia, Chile[2], Institute for Biomedical Research (IRB), Barcelona, España[3].

Glycogen is the main source of glucose for many biological events. However, this molecule may have other functions, including those that have deleterious effects on cells. The rate-limiting enzyme in glycogen synthesis is glycogen synthase (GS). Expression of GS and its activity have been widely studied in many tissues. To date, it is not clear which GS isoform is responsible for glycogen synthesis and the role of glycogen in testis. Using RT-PCR, Western blot and immunofluorescence, we have detected expression of MGS (muscle glycogen synthase) but not LGS (liver glycogen synthase) in mice testis during development. We have also evaluated GS activity and glycogen storage at different days after birth and we show that both GS activity and levels of glycogen are higher during the first days of development. Using RT-PCR and immunoblotting, we have also shown that malin and laforin are expressed in testis, key enzymes for regulation of GS activity. These proteins form an active complex that regulates MGS by poly-ubiquitination in both Sertoli cell and male germ cell lines. In addition, PTG overexpression in male germ cell line triggered apoptosis by caspase3 activation, proposing a proapoptotic role of glycogen In testis. These findings suggest that GS activity and glycogen synthesis in testis could be highly regulated and a disruption of this process may be responsible for the apoptosis and degeneration of seminiferous tubules and possible cause of infertility. FONDECYT-1110508, -1090740; DID-UACh-S-201-14; Catalunya-Spain 2009-SGR-01176, BFU2011-30554; CONICYT-AT2410001; MECESUP-UCO0606, -AUS0704.

Response to inclination in young seedlings of radiata pine. Hormonal signaling and molecular players. Ramos, P.[1], Valenzuela, C.[1], Cruz, N.[1], Moya-León, M.A.[1], Herrera, R.[1]. Laboratorio de Fisiología Vegetal y Genética Molecular, IBVB, Universidad de Talca, Chile[1].

Gravitropic response and the consequent stem reorientation is an attractive and dramatic adaptive behavior of plants from the biological point of view. This response in trees is widely studied phenomenon, but the molecular mechanism is still unknown. The genetic reprogramming involves was studied in one-year-old radiata pine seedlings exposed to inclination. Addressed to characterize this response, whole seedlings were inclined at 45°. First, transversal cuts in order to identify morphological compression wood characteristics on xylem cells by optical microscopy in a time course experiment were performed. In parallel, by a transcriptomic approach, eight Suppressive Subtractive Hybridization libraries (SSH) were generated from total RNA extracted at 2.5, 10, 24 hours and 30 days from the inferior and superior half of the inclined stem. From a total of 3,092 sequences obtained, 2,203 elements were assembled and displaying homology to a public database. Finally, 942 unigenes elements were identified using bioinformatic tools. Due to the loss of verticality, many genes were activated, particularly the groups of genes related to metabolism, signal transduction and cellular transport among the others. Interestingly, the expression of specific secondary metabolic pathway genes was induced after gravistimulation, in a temporal and spatial differential manner along the stem. These findings agreed with the time course of develop of compression wood characteristics on inclined stems. Additionally, the role of ethylene hormone was evaluated. Ethylene treatments show that this hormone might influence the morphological characteristics associated with a transcriptional genetic control. .

Structural characterization and substrate specificity of VpAAT1 protein related to ester biosynthesis in mountain papaya fruit. Morales-Quintana, L.[1], Moya-León, M.A.[1], Herrera, R.[1]. Laboratorio de Fisiología Vegetal y Genética Molecular, Instituto de Biología Vegetal y Biotecnología, Universidad de Talca.[1].

In mountain papaya (*Vasconcellea pubescens*) the aroma is formed mainly by esters. The esterification step is catalyzed by alcohol acyl-transferase enzymes (VpAAT1) that transfer an acyl-CoA to an alcohol. To gain insight about the mechanism of action of VpAAT1, the comparative modeling methodology was used to build the enzyme?s structure. The VpAAT1 structure showed two domains connected by a large crossover loop, with a solvent channel in the center of the structure formed between the two domains. Residues H166 and D170 are described as important for catalytic action, and displayed their side chains towards the central cavity of the channel allowing their interaction with the substrates. The H166A, D170A and D170E mutations were evaluated *in silico*. Significant changes on interaction energy and ligand?s orientation in the solvent channel was found, after molecular docking and molecular dynamics simulations, showing unfavorable interaction energies with different alcohols and acyl-CoA substrates, the predictions obtained were tested through kinetic analysis. Kinetic results showed that the lowest *Km* values were obtained for acetyl-CoA and benzyl alcohol. In addition, the most favourable predicted substrate orientation was observed for benzyl alcohol and acetyl CoA, showing a perfect coincidence between kinetic studies and molecular docking analysis. Finally, in vitro site-directed mutagenesis of showed a loss in the enzymatic activity of VpAAT1 mutant enzyme, confirming the functional role of the residues during the VpAAT1 catalysis. L.M.-Q. acknowledges CONICYT for a Doctoral fellowship. Research supported by Anillo ACT-41 project.

Transcriptome analyses identifies specific intracellular pathways and target genes in gastric cancer. Bizama, C.[1], Felipe, B.[2], Jaime, E.[2], Salvatierra, E.[3], Ana, G.[2], Elmer, F.[4], Eduardo, S.[5], Iván, R.[6], Guillermo, M.[7], Osvaldo, P.[3]. Programa de Doctorado en Ciencias Mención Biología Celular y Molecular Aplicada. Facultad de Ciencias Agropecuarias y Forestales. Universidad de La Frontera, Temuco, Chile. [1], Programa de Doctorado en Ciencias Mención Biología Celular y Molecular Aplicada. Facultad de Ciencias Agropecuarias y Forestales. Universidad de La Frontera, Temuco, Chile[2], Laboratorio de Terapia Celular y Molecular. Fundación Instituto Leloir, Buenos Aires, Argentina. [3], Facultad de Ingeniería, Grupo de Minería de Bio-Datos. Universidad Católica de Córdoba, Argentina.[4], Biotecnología. Facultad de Ciencias Agropecuarias y Forestales. Universidad de La Frontera, Temuco, Chile.[5], Servicio de Patología, Clínica Alemana de Santiago. Facultad de Medicina, Universidad del Desarrollo, Chile.[6], Laboratorio de Terapia Génica. Escuela de Medicina. Universidad Austral. Pilar-Buenos Aires, Argentina. [7]. Sponsored by Gutiérrez, A.

Gastric cancer is the fourth most common cancer and the second leading cause of cancer-related death worldwide. Its high mortality rate is associated with the absence of symptoms in the early stage of the disease, leading to a diagnosis in advanced stage. Despite advances in diagnostic imaging that improved early detection of gastric cancer advanced stages have still a poor prognosis. Here we applied conventional microarray analysis and subtractive hybridization (SSH) followed by microarray analysis in order to identify high and low abundance transcripts that were differentially expressed in gastric adenocarcinomas compared with their paired adjacent non-cancerous tissues. Using both strategies we identified a set of genes that was differentially expressed and that were able to discriminate perfectly cancerous gastric tissues compared with adjacent non-cancerous tissues. Differentially expressed genes were associated to cell interaction with the surrounding stroma and with the extracellular matrix. Interestingly, genes detected by the SSH strategy several rare transcripts with unknown functions and genes with other biological functions such as signal transduction and intracellular oncogenic pathways Through this integration we identified several genes that may be critically important for gastric cancer, with potential diagnostic and therapeutic implications. This work was supported by PIA CTE-06 and CONICYT Fellowships.

TMBIM3/GRINA is a novel unfolded protein response (UPR) target gene that controls apoptosis through the modulation of ER calcium homeostasis. Rojas-Rivera, D.[1,2,3], Armisen, R.[2], Colombo, A.[1], Martínez, G.[1,2,3], Eguiguren, A.L.[2], Díaz, A.[1], Kiviluoto, S.[4], Rodríguez, D.[1,2,3], Patron, M.[5], Rizzuto, R.[5], Bultynck, G.[4], Concha, M.[1], Sierralta , J.[1], Stutzin, A.[2], Hetz, C.[1,2,3]. Biomedical Neuroscience Institute, Faculty of Medicine, University of Chile[1], Center for Molecular Studies of the Cell, Institute of Biomedical Sciences, University of Chile, Santiago, Chile[2], Neurounion Biomedical Foundation, Santiago, Chile[3], Katholieke Universiteit Leuven[4], Universita degli Studi di Padova[5]. Sponsored by Lavandero, S.

Transmembrane BAX inhibitor motif-containing (TMBIM)-6, also known as BAX-inhibitor 1 (BI-1), is an anti-apoptotic protein that belongs to a putative family of highly conserved and poorly characterized genes. Here we report the function of TMBIM3/GRINA in the control of cell death by endoplasmic reticulum (ER) stress. Tmbim3 mRNA levels are strongly upregulated in cellular and animal models of ER stress, controlled by the PERK signaling branch of the unfolded protein response. TMBIM3/GRINA synergies with TMBIM6/BI-1 in the modulation of ER calcium homeostasis and apoptosis, associated with physical Interactions with inositol trisphosphate receptors, which could explain its anti-apoptotic activity under conditions of ER stress. Loss-of-function studies in D. melanogaster demonstrated that TMBiM3/GRINA and TMBIM6/BI-1 have synergistic activities against ER stress in vivo. Similarly, manipulation of TMBIM3/GRINA levels in zebrafish embryos revealed an essential role in the control of apoptosis during neuronal development and in experimental models of ER stress. These findings suggest the existence of a conserved group of functionally related cell death regulators across species beyond the BCL-2 family of proteins operating at the ER membrane.Funding: FONDECYT 1100176 FONDAP grant 15010006, ACT1109, Millennium Institute No. P09-015-F, Michael J. Fox Foundation for Parkinson Research. Muscular Dystrophy Association, ALS Therapy Alliance, Capital Humano en la Academia 79100007, CONICYT Doctoral fellowship (DR-R); UCH-0606 (DR-R); CONICYT no.24090143 (DR-R).

ORAL SESSIONS

STRUCTURE AND FUNCTION OF MACROMOLECULES

RNA aptamers capable of inhibiting isoform LI2 of spider venom sphingomyelinase D (SMD-LI2). **Salinas-Luypaert, C.**[1], Sapag, A.[1]. Laboratory of Gene Pharmacotherapy, Department of Pharmacological and Toxicological Chemistry, Faculty of Chemical and Pharmaceutical Sciences, Universidad de Chile, Santiago, Chile[1].

Sphingomyelinase D is the main toxin found in the venom of *Loxosceles spp.* spiders, making this phospholipase a target for developing both antidotes against the envenomation caused by the spider bite (loxoscelism) and diagnostic means of the disease. Aptamers are oligonucleotides capable of binding tightly and specifically to their target and inhibiting their biological function. RNA aptamers which bind to SMD-LI2, the most toxic sphingomyelinase D isoform of *Loxosceles laeta*, were obtained by iterative selection from a pool of random sequence molecules. The SMD-LI2 cDNA was synthesized by PCR oligonucleotide assembly and cloned in a plasmid for expression in *Escherichia coli*. Recombinant SMD-LI2 protein was produced with an N-terminal histidine tag and purified in active form (154 U/mg) by affinity chromatography. A random oligonucleotide pool (60 Ns in the center) was amplified by PCR and transcribed to generate a universe of $>10^{13}$ different RNAs (107 nucleotides long). Aptamers capable of binding to SMD-LI2 were selected from these RNAs by performing 12 rounds of *in vitro* selection by filtration through nitrocellulose membranes. The ability of all 12 RNA sets to inhibit sphingomyelinase activity was measured in a fluorimetric assay. Group twelve was cloned and 52 clones were sequenced. Nine sequence families with <50% inter family identity and >92% intra family identity were found. The inhibition capacity of 19 individual aptamers, representing all families, was assessed. Two aptamers of different families displayed significant inhibition of SMD-LI2 activity, 26% and 23%. (FONDECYT 1100209).

A mechanism for the substrate inhibition of pig kidney fructose-1,6-bisphosphatase: mixed-subunit oligomers and tryptophan-containing mutants allow two different classes of binding sites to be distinguished. **Asenjo, J.L.**[1], Ludwig, H.C.[1], Droppelmann, C.A.[1], Cárcamo, J.G.[1], Cárdenas, M.[2], Concha, I.I.[1], Yáñez, A.E.[1], Cornish-Bowden, A.[2], Slebe, J.C.[1]. Instituto de Bioquímica y Microbiología, Universidad Austral de Chile.[1], Bioénergétique et Ingénierie des Protéines, Institut de Microbiologie de la Méditerranée, CNRS, Aix-Marseille Université, Marseilles, France[2].

Fructose-1,6-bisphosphatase, a key regulatory enzyme in gluconeogenesis, is a homotetramer which does not contain tryptophan. It has long been known that fructose-1,6-bisphosphatases isolated from different sources are inhibited by their substrate, but the mechanism of the inhibition has remained obscure. Three types of experiments were used to shed light on this: (1) fluorescence studies with mutants in which phenylalanine residues were replaced by tryptophan; (2) exchange of subunits between wild-type and Glu-tagged oligomers; (3) kinetic measurements over a very wide range of substrate concentrations, subjected to detailed statistical analysis. At concentrations near the K_M value of 4 μmolar, the substrate Fru-1,6-P_2 caused an increase in the intrinsic fluorescence of the Phe219Trp enzyme whereas almost no perturbation was observed when the Phe232Trp enzyme was titrated. The transition was well described by a single binding isotherm, with a K_d of 7.9 μmolar. The overall results indicate that two different classes of catalytic sites exist that differ greatly in their affinity for substrate. Binding of substrate to the low-affinity sites act as a "stapler" that prevents dissociation and hence exchange of subunits. The substrate inhibition results from the binding of fructose-1,6-bisphosphate to the low-affinity sites. (Acknowledgements: Fondecyt 1090740; Fellowship (JLA) AUS0006, DID-UACH).

Atomic model of the core of *Gracilaria chilensis* phycobilisome. **Dagnino-Leone, J.**[1], Figueroa, M.[2], Bunster, M.[1], Martínez-Oyanedel, J.[1]. Laboratorio de Biofísica Molecular, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción[1], GIGA-Research, Molecular Biology and Genetic Engineering Unit, University of Liege. Belgium.[2].

Phycobilisomes (PBS) are light-harvesting complexes present in cyanobacteria and red algae. The PBS are composed of three phycobiliproteins: phycoerythrin (PE), phycocyanin (PC), allophycocyanin (APC) and linkers proteins. The basic unit of the phycobiliproteins is a heterodimer (αβ) which oligomerizes as trimers $(ab)_3$ and hexamers $(\alpha\beta)_6$.PBS has 2 components identifiable by electron microscopy studies, rods and a core: rods are cylinders composed of phycoerythrin and phycocyanin. The core is composed of APC cylinders.There are not atomic models of PBS. We have resolved the structure of PE and PC from *Gracilaria chilensis*, and for APC we have sequenced the genes. The translated sequence was used to build a 3D model for the subunits and trimers of APC using Modeller. Then a molecular docking using CLUSPRO was performed to obtain hexamer models according to results obtained by gel filtration chromatography. The models were submitted to 1 ns molecular dynamics (MD) using GROMACS. 6 APC hexamers were used to build a three-cylinder model for core of the PBS of *G.chilensis*. The model was submitted to 5 ns MD to relax its structure. To determine the preferential energy transfer pathways in the Core model, we applied the Forster equation. Our results show that there are energy transfers between adjacent hexamers with transfer time near to 200 ps and longitudinal energy transfer between hexamers with transfer time near 30 ps. Supported by Diuc 211.37.12-1.

Towards deciphering the protein-DNA recognition code by statistical analysis of amino acid-base contact propensities. **Schüller, A.**[1,2], Melo, F.[1,2]. Molecular Bioinformatics Laboratory, Millennium Institute on Immunology and Immunotherapy[1], Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile[2].

Protein-DNA interactions play pivotal roles in many biological processes such as DNA transcription, replication, repair, and their regulation inside cells. Specific recognition of DNA binding sites is thought to be governed by two mechanisms: amino acid-base interactions (base readout) and detection of DNA shape (shape readout). Towards this end we are particularly interested in understanding the specific recognition code of helix-turn-helix type transcription factors (HTH-TFs), an important class of transcriptional regulators known to recognize DNA by both principles. We have analyzed the contact preferences of 208 protein-DNA complexes derived from a non-redundant subset of our protein-DNA interface database. A total of 48,731 contacts were detected on an atomic level in the three-dimensional protein-DNA structures applying explicit angular and distance constraints. Our results indicate a particular bias of HTH-TFs to interact with thymine via specific hydrophobic contacts. Hydrophobic amino acid-thymine contacts accounted for 77% of all major groove interactions in the case of HTH-TFs, while the number was 46% in all other cases. We further observed an approximately 2.5-fold increase in contacts of amino acids with two adjacent adenine-thymine and thymine-thymine dinucleotides. Poly-adenine (poly-thymine) tracks are known to increase intrinsic bending of DNA, while alternating poly-AT tracks are known to increase DNA flexibility. We suggest that HTH-TFs recognize their DNA binding sites by preferential thymine base readout and by shape readout of thymine-induced DNA curvature. Acknowledgments: FONDECYT 3110009 to A.S., FONDECYT 1110400 and ICM P09-016-F to F.M.

Structure-based prediction of transcription factor binding specificities using a Metropolis-Montecarlo simulation approach. Norambuena, T.[1,2], Melo, F.[1,2]. Molecular Bioinformatics Laboratory, Millennium Institute on Immunology and Immunotherapy[1], Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.[2].
Protein-DNA binding is of utmost importance since it is involved in cellular processes such as gene expression and cell division. Since the first DNA-protein structure complex was solved at atomic resolution, our knowledge about how the recognition is carried out has increased notably. Several studies have been reported, where sequence as well as structural information has been used to predict protein-DNA binding specificities. Despite sequence-based methods are widely used, they are still not very accurate, exhibiting poor sensitivity/specificity trade offs.We have developed a structure-based method for predicting binding sites in the DNA. This method relies on software for the 3D modelling of protein-DNA complexes and on statistical potentials, which estimate the protein-DNA stability from the 3D structure. We have included this approach in a protocol for predicting specificities of transcription factors at the DNA level. The core of the protocol is a Metropolis-Montecarlo simulation, a random sampling method for modelling systems with many coupled degrees of freedom, that allows us to reduce the sampling space as well as to obtain DNA sequences that bind with a medium-high or high affinity.Here we report this protocol and show that we can obtain ensembles of DNA sequences with high fitness for the protein-DNA complex under study and, at the same time, recover in a large degree the known experimental specificities for several transcription factors. ACKNOWLEDGEMENTSThis research was funded by grants from FONDECYT (3120007) and ICM (P09-016-F).

Comparative modeling of B-DNA structures. Ibarra, I.[1,2], Madhusudhan, M.[3], Melo, F.[1,2]. Molecular Bioinformatics Laboratory, Millennium Institute on Immunology and Immunotherapy[1], Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile[2], Biomolecular Modeling and Design Division, Bioinformatics Institute, 30 Biopolis Street, Singapore[3].
Few tools for full atom three-dimensional modeling of nucleic acids are currently available. Besides, when the stereochemical accuracy of the generated models is assessed, structural defects are identified. The reason is that most of currently available tools mainly focus in the modeling of base pairs and not in the sugar phosphate backbone, which is relevant for protein-DNA complexes modeling. In this work, a comparative modeling tool for building of full atom B-DNA models using similar structures has been developed. The protocol generates models with good stereochemistry and preserves the model conformation as close as that observed for the template, while optimizing conformational changes generated by base replacements. It can be coupled with different applications and with more molecules. For the development of this tool we have used isolated DNA structures solved by X-ray crystallography. We extracted 87 B-DNA structures from the Nucleic Acid Database and classified them into two groups: 1) a training set (76) to derive structural restraints, and 2) a testing set (11), for the performance assessment of the method in the creation of B-DNA models. All the target-template permutations of the testing set were modeled, obtaining 255 structures to evaluate the model quality. We suggest that this improved modeling protocol for DNA molecules will be also useful for the three-dimensional modeling of protein-DNA complexes, helping to understand the affinity and specificity rules that underlie this molecular recognition process. Acknowledgments: This work is funded by FONDECYT (1110400).

RNABPviewer, a software tool for the three-dimensional analysis and visualization of canonical and non-canonical base pairs in RNA structures. Cares Galvez, J.[1,2], Rodríguez, F.[1,2], Melo, F.[1,2]. Molecular Bioinformatics Laboratory, Millennium Institute on Immunology and Immunotherapy, [1], Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile.[2].
Classically, RNA molecules are given the title of biological information carrier used in the synthesis of proteins and ribosomal structural cores. Lately, new RNA functions are being discovered, like their biological activity as regulators and catalysts, and their role in cell signaling. To better understand the function of RNA molecules, the study of their three-dimensional structures is required. Features like canonical and non-canonical base pairing and base stacking are essential driving forces that stabilize this molecule. The main focus of this work is to improve the base pairing visualization in RNA structures using PyMOL, a well-known molecule 3D viewer, and the software RNAView, a tool for identifying base pairs in RNA structures. Towards that end, we have developed a plugin that changes the molecule coloring and representation based on a base pairing nomenclature proposed by Leontis and Westhof . Additionally, this tool shows a table detailing the base pair counting in the selected structure.We expect this tool will be useful for the analysis of the growing number of RNA structures available at the PDB.This research was funded by grants from FONDECYT (1110400) and ICM (P09-016-F).

Energy and structural characterization of the interaction between Smac/DIABLO N-terminal peptides and human Survivin using bioinformatics tools. Acevedo, W.[1], Guzmán, L.[1], Aguilar, L.F.[1]. Instituto de Química, Pontificia Universidad Católica de Valparaíso. Avenida Universidad #330. Campus Curauma, Valparaíso.[1]. Sponsored by Guzmán, L.
Survivin is selectively overexpressed in most human cancers but in not normal tissues. Furthermore, it is a member of the inhibitor-of-apoptosis (IAP) family of proteins, whose studies of NMR spectroscopy have been used to determine the homodimer interface and to identify residues of the protein that interact with Smac/Diablo. This work has as propose to characterize the interaction between Smac/Diablo peptides analogues and Survivin. For the study, it used the complete crystallography structure of human Survivin. To perform the theoretical calculations initially were built analogues peptide to SMAC with gaussview code from the first 20 amino acids. With this method were designed 9 peptides with different amounts of residues (between 4 and 20). For each structure built docking calculations were performed, using Autodock 4.0 code, to assess the interaction between peptides analogous to SMAC and Survivin based on the interaction free energies. Subsequently, we selected the peptide with higher affinity and molecular simulation was performed between the selected peptide and survivin by NAMD code. From the theoretical results we have concluded that those sequences with more hydrophobic residues have a greater interaction with survivin. Furthermore, by software analysis used let us characterize in detail how analogous N-terminal Smac/Diablo peptides interact with Human Survivin. Acknowledgements: This work was supported by Dirección de Investigación e Innovación of the Vicerrectoría de Investigación y Estudios Avanzados; project DII 037.427/2012.

MOLECULAR BIOLOGY OF THE CELL I

Establishment of primary cultures of advanced cervical cancer as a model for antisense therapy. Avila, R.[1], Socias, M.[2], Dadlani, K.[3], Zapata, L.[4], Villota, C.[5], Socias, T.[6], Oliveira-Cruz, L.[6], Burzio, V.[7], Bustamante, E.[8], Burzio, L.[5], Villegas, J.[5]. Fundación Ciencia para la Vida, Andes Biotecnologies S.A., Fac. Ciencia Biológicas, Universidad Andrés Bello[1], Clínica Alemana[2], Fundación Ciencia para la Vida[3], Anatomía Patológica, Hospital Barros Luco Trudeau[4], Fundación Ciencia para la Vida, Andes Biotechnologies S.A., Fac. Ciencias Biológicas, Universidad Andrés Bello[5], Fundación Ciencia para la Vida, Andes Biotechnologies S.A.[6], Fundación Ciencia para la Vida, Andes Biotecnologies S.A., Fac. Ciencias Biológicas, Universidad Andrés Bello[7], Fundación Arturo López Pérez[8].

Cervical cancer is the second most common cancer in women. It is estimated that about 530,000 women are diagnosed each year and about 50% die of this disease. Therefore, is necessary to develop effective therapies with low side effects. In our laboratory we described a family of noncoding mitochondrial RNAs (ncmtRNAs), comprised of sense and antisense variants, which show differential expression depending on the cell proliferative status. Normal proliferating cells in culture, as well as biopsies, show the presence of sense and antisense molecules, resting cells have a low presence of both molecules and in tumor cells only the sense is detected. We show that treatment of tumor cell lines derived from cervical cancer (He-La and SiHa) with antisense oligonucleotides (ASO) against the antisense molecule triggers massive death by apoptosis, (about 80%). ASO treatment does not induce transformation events or death in normal cells, indicating biosafety. We established primary cultures from advanced cervical cancer biopsies, and tumor cells were selected using the colony formation assay and characterized using antibodies againts p16INK4a, cytokeratin 17, EpCAM and survivin. For ASO treatment, we isolated and characterized colonies 50-80 μm in diameter. Determination of the oligo uptake was performed with different transfection agents and fluorescent oligonucleotides. The effect of ASO treatment on cell viability of primary cultures was assessed by MTT assays, TUNEL and the reduced ability to form colonies in soft agar. FONDEF D10I1090, CCTE-PFB-16 Grants, CONICYT, Chile.

A bidirectional and complex relationship between mitochondrial dynamics and insulin signaling in skeletal muscle cells. del Campo, A.[1], Parra, V.[1], Gutiérrez, T.[1], Morales, P.E.[1], Lavandero, S.[1,2,3]. Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/Faculty of Medicine[1], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile. [2], Department of Internal Medicine, University of Texas Southwestern Medical Center, USA.[3].

Insulin is the major regulator of the metabolic response after nutrient consumption. This hormone promotes a metabolic switch towards the uptake and use of glucose in skeletal muscle, which is essential to maintain glucose homeostasis. On the other hand, mitochondria are the organelles in charge of driving glucose to ATP through the Krebs cycle. Both, insulin and mitochondria are then *tightly united to control cell metabolism*. However the relationship between mitochondrial dynamics and insulin signaling its almost unknown. Our data show that insulin stimulates mitochondrial activity enhancing oxygen consumption after 3 h stimuli. This response was accompanied by increases in mitochondrial fusion and the levels of mitochondrial protein OPA1, a critical protein for fusion processes and cristae remodeling of mitochondria. We also depict that changes in mitochondrial morphology induced by adenovirus or miRNA against Mfn2 and Opa1, promote diminished insulin signaling reflected in a decrease of Akt phosphorylation and glucose uptake. On the other hand, we also found that insulin promotes an increase in mitochondrial calcium movements, effect that is delayed and diminished by mitochondrial fission.

Unveiling molecular constituents of a multiprotein complex involved in excitation-transcription coupling in skeletal Muscle. Buvinic, S.[1,2], Almarza, G.[2], Arias, M.[2], Jaimovich, E.[2]. Departamento de Ciencias Básicas y Comunitarias, Facultad de Odontología, Universidad de Chile, Santiago, Chile.[1], Centro de Estudios Moleculares de la Célula, ICBM, Facultad de Medicina, Universidad de Chile, Santiago, Chile.[2].

Electrical activity regulates the expression of skeletal muscle genes by a process known as "excitation-transcription" (E-T) coupling. We have demonstrated that release of ATP during depolarization activates membrane P2X/P2Y receptors, being fundamental mediators between electrical stimulation, slow intracellular calcium transients and gene expression. We propose that this signaling pathway would require the proper coordination between the voltage sensor (Cav1.1, dihydropyridine receptor, DHPR), pannexin1 hemichanel (PnX1, ATP release conduit), nucleotide receptors, and several signaling molecules. The goal of this study was to assess protein interactions between the E-T machinery, and looking for novel constituents in order to characterize the signaling complex. Newborn derived myotubes, adult fibers or triad fractions from rat or mouse skeletal muscles were used. PnX1, DHPR, $P2Y_2$, and dystrophin (dys) co-immunoprecipitated in the different preparations assessed. Using the proximity ligation assay associated to confocal microscopy, we observed co-localization between DHPR, PnX1, $P2Y_2$ and caveolin3 (cav3) in adult fibers. Using 2D blue-native SDS/PAGE we detected that DHPR, PnX1, $P2Y_2$, Cav3, dys and Ryanodine receptor belong to the same multiprotein complex. Novel constituents derived from that complex are actually being isolated by blue-native SDS/PAGE or Strep-tag purification and analyzed by mass spectrometry. Several proteins involved in the E-T coupling appear to belong to a multiprotein complex for fine-tuning gene expression. Unveiling molecular actors involved in muscle plasticity could contribute to the understanding and treatment of skeletal muscle disorders such as distrophies or sarcopenia associated with aging. Fondecyt-1110467-11100454, Conicyt-79090021, FONDAP-15010006, ACT-1111.

Herp cytoprotective effect against oxidative stress through regulation of the inositol trisphosphate receptor (IP3R). Paredes, F.[1], Gatica, D.[1], Quiroga, C.[1], Parra, V.[1], Bravo, R.[1], Contreras, A.[1,2], Troncoso, R.[1], Jaimovich, E.[1,2], Lavandero, S.[1,3]. Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/Faculty of Medicine[1], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile[2], Department of Internal Medicine, University of Texas Southwestern Medical Center, USA[3].

Herp is an endoplasmic reticulum (ER) protein expressed in response to ER stress, osmotic stress or deregulation of Ca^{2+} homeostasis. Herp exerts cytoprotective effect against some stressors by regulating intracellular calcium. It is still unknown whether this response is also activated by other types of stress and the specific signaling pathways involved in this effect. Our group has found that Herp is also induced *in response to oxidative* stress, reaching its maximal expression (6 fold) within 30 min after HeLa cells or MEF cells were treated with H_2O_2 500 uM. Cell death, assessed by PI and flow cytometry, was significantly higher in Hela cells knock down for Herp (Sh-Herp) and treated with 500 uM H_2O_2 in comparison with control cells (Sh-Luc). This effect was dependent of the presence of intracellular calcium stores increases since it is inhibited with BAPTA-AM. Intracellular calcium kinetics using Fluo-4 shows that Sh-Herp HeLa cells exhibit a greater and more sustained increase over time that Sh-Luc HeLa cells. This effect depends on the IP3R, which was checked using XeB. On the other hand, Sh-Herp Hela cells incubated with Ruthenium-Red or oligomycin were more sensitive to metabolic changes because mitochondrial potential and cellular respiration decreased, leading to an increased cell death Interestingly, cell viability recovered when cells knowdown for Herp were treated with methyl-pyruvate before exposure to 500 uM H_2O_2.Supported by Anillo ACT1111 (EJ and SL) and CONICYT (FP). FP and RB hold a CONICYT fellowship.

Effect of testosterone and insuline on unfolded protein response (UPR) and glucose uptake in cultured human endometrial cells. **Rosas, C.**[1], Poblete, C.[1], Romero, C.[2,3], Lavandero, S.[4], Vega, M.[2,3]. Endocrinology and Reproductive Biology Laboratory, University of Chile Clinical Hospital[1], Endocrinology and Reproductive Biology Laboratory, University of Chile Clinical Hospital.[2], Obstetric/Ginecology Department, University of Chile Clinical Hospital.[3], Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/ Faculty of Medicine, University of Chile. [4].
Hyperandrogemia and hyperinsulinemia are associated with higher miscarriage rate in women with Polycystic Ovarian Syndrome (PCOS) with insulin-resistance, probably reducing the energetic availability in human endometrium. This could be related with endoplasmic reticulum (ER) homeostasis, as described in experimental models where insulin signaling has been assessed. We evaluate whether long exposure of testosterone or insulin affects UPR activity and 2-deoxy-glucose uptake in immortalized human endometrial stromal cells. Cells were maintained in 2% steroids-free serum medium with testosterone or insulin, 100 nM each by 24 and 48-h. Next, the cultures were challenged to 20-min insulin 100 nM prior to 5-min [H3]-2-deoxy-D-glucose-pulse. On the other hand, cells from the same passage were homogenized for Western-blot analysis of phospho-Akt-Ser473 (an insulin-induced PI3K/Akt pathway), phospho-JNK-Thr183/Tyr185 (an UPR-activated Insulin-Receptor/IRS downregulator) and GRP78 (an ER-stress marker). The results show a 50% decrease of glucose uptake in cells exposed to testosterone for 48-h, compared with basal and insulin-stimulated cells ($p<0.05$). The protein levels of phospho-Akt-Ser473 and phospho-JNK-Thr183/Tyr185 are similar between testosterone and insulin exposed cultures at 24 or 48-h. However, testosterone-treated cells exhibit 30% lesser GRP78 content at 24 and 48-h, compared with basal. Inversely, long exposure to insulin (24 and 48-h) leads to similar levels of GRP78 protein with controls. The results suggest that UPR activity is differentially regulated by these hormones and UPR could be involved in glucose availability in PCOS women endometria.Funded by: FONDECYT 1095127-(MV), FONDAP 15010006 and ACT1111-(SL), Doctoral thesis CONICYT grant 24121256-(CR).

Functional evidence reveals two clathrin light chains in *Arabidopsis thaliana*. **Vásquez, B.**[1], Urrutia, P.[1], Norambuena, L.[1]. Laboratorio de Biología Molecular Vegetal, Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile[1].
Vesicular transport is a cellular component that allows the proper destination of proteins within the cell. Clathrin coated vesicles (CCV) mediate endocytosis and trafficking from Trans-Golgi to prevacuolar compartments. Clathrin structure corresponds to three heavy and three light chains (CLC). CLC has an important regulatory role in the formation of CCV. CLC physiological functions have been predominantly described in yeast and mammalian cells. However, less is known about CLC function in plants. Three putative CLCs have been proposed in *Arabidopsis thaliana* (AtCLCs). Only AtCLC2 has been studied by colocalization and interaction assays with proteins that mediate CCVs formation. Considering that functional studies of isolated genes are difficult in plants since gene redundancy, we decided to test functionally two AtCLC using a heterologous system. We studied the ability of both proteins to complement defective phenotypes of the *Saccharomyces cerevisiae* deletion mutant, clc1. Both proteins were able to rescue the slower endocytosis rate in clc1 strain. Moreover, AtCLC1 and AtCLC2 significantly abolished the abnormal vacuolar phenotype of clc1 yeasts. However neither AtCLC were able to revert the defective growth rate of clc1 strain showing physiological difference to the yeast CLC. Interestingly AtCLC1 and AtCLC2 migrate with a higher molecular weight than theoretically predicted in a SDS-PAGE, similar to other CLCs. Overall, our results show that both AtCLC1 and AtCLC2 behave as CLC, and strongly suggest the existence of two functional CLCs in Arabidopsis. Acknowledgements to FONDECYT 1120289 and 11080240.

Methylation status of tumor suppressor genes in total DNA sputum samples: a promising tool for early detection of COPD and lung cancer in smokers. **Guzmán, L.**[1], Depix, M.S.[2], Salinas, A.M.[2], Roldán, R.[3], Aguayo, F.[4], Alejandra, S.[2], Vinet, R.[5,6]. Instituto de Química, Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile[1], Facultad de Salud, Escuela de Tecnología Médica, Universidad Santo Tomás, Santiago, Chile[2], Unidad de Enfermedades Respiratorias, Hospital San José, Santiago, Chile[3], Programa de Virología, Instituto de Ciencias Biomédicas (ICBM), Facultad de Medicina, Universidad de Chile, Santiago, Chile.[4], Facultad de Farmacia, Universidad de Valparaíso, Valparaíso, Chile.[5], Centro Regional de Estudios en Alimentos Saludables (CREAS), Valparaíso, Chile.[6].
Chronic obstructive pulmonary disease (COPD) is a disorder associated to cigarette smoke and lung cancer (LC). Since epigenetic changes in oncogenes and tumor suppressor genes (TSGs) are clearly important in the development of LC. In this study, we hypothesize that tobacco smokers are susceptible for methylation in the promoter region of TSGs in airway epithelial cells when compared with non-smoker subjects. The purpose of this study was to investigate the usefulness of detection of genes promoter methylation in sputum specimens, as a complementary tool to identify LC biomarkers among smokers with early COPD.We determined the amount of DNA in induced sputum from patients with COPD (n=23), LC (n=26), as well as in healthy subjects (CTR) (n=33). The frequency of CDKN2A, CDH1 and MGMT promoter methylation in the same groups was determined by methylation-specific polymerase chain reaction (MSP). The Fisher's exact test was employed to compare frequency of results between different groups.DNA concentration was 7.4 and 5.8 times higher in LC and COPD compared to the CTR ($p<0.0001$), respectively. Methylation status of CDKN2A and MGMT was significantly higher in COPD and LC patients compared with CTR group ($p<0.0001$). Frequency of CDH1 methylation only showed a statistically significant difference between LC patients and CTR group ($p<0.05$). We provide evidence that aberrant methylation of TSGs in samples of induced sputum is a useful tool for early diagnostic of lung.

Dexamethasone stimulates autophagy and metabolic changes in skeletal muscle cells. **Troncoso, R.**[1], Paredes, F.[1], Gatica, D.[1], Vasquez-Trincado, C.[1], Rodríguez, A.E.[1], Lavandero, S.[1,2,3]. Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/Faculty of Medicine, University of Chile[1], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile[2], Department of Internal Medicine, University of Texas Southwestern Medical Center, USA[3].
Glucocorticoids (GCs) regulate growth, metabolism, development and immune functions and play a pivotal role in preserving basal and stress-related homeostasis. In skeletal muscle, GCs induce atrophy and insulin resistance. Autophagy is a degradative pathway for cellular components and has an essential role in preserving skeletal muscle mass. We studied the effect of the GC dexamethasone (DEX) on autophagy and mitochondrial metabolism in rat L6 skeletal muscle cells. Autophagy was assessed by the formation of endogenous LC3 dot by immunofluorescence and by the processing of LC3-I to LC3-II by Western blot (WB). p62 levels and phosphorylated forms of mTOR, AMPK and Akt were also determined by WB. Mitochondrial metabolism was evaluated by mitochondrial membrane potential (ψmt), reactive oxygen species (ROS), oxygen consumption and ATP levels. Our results show that DEX for 6 h stimulated redistribution and processing of LC3 and decreased p62 levels. DEX also stimulated autophagy flux and this effect was blunted by a short-hairpin for Beclin-1. Moreover, DEX for 24 h induced the expression of LC3 and p62. On the other hand, DEX for 24 h changed mitochondrial metabolism, decreasing mitochondrial metabolism (ψmt, ROS, oxygen consumption) and increased ATP levels. Our data suggest that DEX the biphasic response induced by DEX that involved early autophagy induction and later mitochondrial metabolic changes. Supported by Anillo ACT1111 and FONDECYT 1120212 (SL) and FONDECYT 3110114 (RT). FP, AR, CV hold a CONICYT fellowship.

PROTEIN STRUCTURE AND FUNCTION

Salsolinol and isosalsolinol: products of dopamine and acetaldehyde condensation as final effectors of the reinforcing effect of ethanol. Berríos-Cárcamo, P.A.[1], Rivera-Meza, M.[2,3], Buscaglia, M.[2], Zapata-Torres, G.[4], Herrera-Marschitz, M.[3], Israel, Y.[1]. Laboratorio de Farmacoterapia Génica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile[1], Laboratorio de Farmacogenética del Alcoholismo, Facultad de Medicina, Universidad de Chile[2], Biomedical Neuroscience Institute (BNI), Programme of Molecular & Clinical Pharmacology, Institute of Biomedical Sciences (ICBM), Faculty of Medicine, University of Chile[3], Unidad de Gráfica Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile[4]. Sponsored by Sapag, A.
Acetaldehyde is the reinforcing metabolite of alcohol. Salsolinol, a Pictet-Spengler condensation product of acetaldehyde and dopamine, can also be involved in the mechanism of ethanol reinforcement. Recent studies report that salsolinol has reinforcing properties, likely via mu-opioid receptors. The referred studies used *Sigma-Aldrich* salsolinol, comprising the analogs (+-)-salsolinol and (+-)-isosalsolinol (secondary product of dopamine and acetaldehyde condensation); which of the analogs is the active product remains to be studied. To evaluate whether a salsolinol analog is related to the reinforcing effect of ethanol, we studied (i) the binding (docking) of salsolinol analogs to a mu-opioid receptor *in silico*; (ii) the synthesis of salsolinol analogs *in vitro*; and (iii) the effect of salsolinol analogs in vivo in rat brain, determining the active concentrations. (i) Docking analyses showed that all salsolinol analogs fit into the morphine pocket of the mu-opioid receptor. (ii) Only a non-enzymatic pathway could be characterised, the velocity constants being 29 and 1.8 (1/Mmin) for salsolinol and isosalsolinol, respectively, estimating a synthesis rate from the concentrations of synaptic dopamine and acetaldehyde derived from ethanol. (iii) *In vivo* salsolinol exerts its effects in the sub-uM range, requiring about 10 min for its synthesis. In conclusion, active salsolinol may be synthetized in vivo. Its importance for the reinforcing effect of ethanol likely depends on its accumulation to relevant concentrations in the proper brain locus. Acknowledgements: FONDECYT-grants, Chile, #1095021 (YI), #1120079 (MH-M) and #3110107 (MRM).

Role of the electrostatic potential in the cooperativity between pHo-sensors in K2P channel TASK-3. González, W.[1], Arévalo, B.[1], Zúñiga, L.[2], Niemeyer, M.I.[2], Cid, P.[2], Sepúlveda, F.[2]. Centro de Bioinformática y Simulación Molecular (CBSM), Universidad de Talca[1], Centro de Estudios Científicos (CECs)[2].
Two-pore domain potassium (K2P) channels are membrane proteins identified in mammals and other organisms. The functional channel is a dimer and each subunit has two pore-forming loops and four transmembrane domains. In mammals, fifteen KCNK genes have been identified, and their codified proteins (K2P potassium channels) are related with several pathologies in humans. Among K2P channels, those belonging to the TASK subfamily such as TASK-1 and TASK-3 are sensitive to extracellular proton concentration. TASK-3 is closed at acid extracellular pH (pHo) but it is open when pHo increased. The pHo-sensor is a histidine residue (H98) placed above the selectivity filter (sequence GYGH). When one H98 is protonated, the potassium ion (K+) placed in the S0 site of the selectivity filter is rapidly destabilized. The loss of K+ placed on S0, diminishes the positive electrostatic potential around the second H98 requiring less energy for its protonation. This mechanism is in agreement with experimental measurements of pKa reporting that protonation of the sensor in one subunit enhances the propensity of that in the second to become protonated.Acknowledgments: Fondecyt 11100373.

Caveolin-1 reduces HIF1a dependent transcription and target gene expression in cancer cells via a mechanism involving nitric oxide. Sanhueza, C.[1], Silva, P.[1], Diaz, M.I.[1], Ávalos, Y.[1], Leyton, L.[1], Quest, A.[1]. Laboratorio de Comunicaciones Celulares, Centro de Estudios Moleculares de la Célula, Facultad de Medicina, Universidad de Chile[1].
Stabilization of HIF1a due to suppression of proteasome-mediated degradation under hypoxic conditions observed in solid tumors promotes tumor growth. Caveolin-1 (Cav-1) is a scaffolding protein that often inhibits signal transduction cascades important for cell proliferation and survival via interactions involving the Caveolin-Scaffolding-Domain (CSD). Alternatively, results from this laboratory showed that Cav-1 promotes proteasome-mediated degradation of the inducible isoform of nitric oxide synthase (iNOS) via a CSD-independent mechanism. How such mechanisms contribute to Cav-1 function as tumor suppressor remain poorly understood. Here, we evaluated the possibility that Cav-1 may suppress HIF1a transcriptional activity by precluding nitric oxide dependent HIF1a stabilization.HT29(US) colon, B16F10 melanoma cancer cells expressing or not Cav-1 were exposed to hypoxia for 24 h. HIF-1 transcriptional activity, target-gene expression, HIF1a localization and protein levels were detected by gene-reporter assays, RT-PCR, qPCR, confocal microscopy and Western blot assays, respectively. Cav-1 overexpression reduced HIF1-dependent transcriptional activity, target-gene expression and HIF1a nuclear protein content in hypoxia. *In vivo*, VEGF a target-gene of HIF1a was reduced in tumors expressing Cav-1. No colocalization was detected between Cav-1 and HIF1a in hypoxia. Interestingly, L-NAME (NO synthase inhibitor) reduced hypoxia-induced HIF1 activity observed in cells lacking Cav-1 to levels detected in Cav-1 expressing cells in hypoxia. The results suggest that Cav-1 reduces HIF-1a activity and target-gene expression in hypoxia by reducing NO production, possibly via a mechanism involving iNOS.CONICYT (CS, PS, YA), AFGQ (FONDAP 15010006, FONDECYT 1090071, ACT1111), LL (FIRCA 5R03TW007810-2, FONDECYT 1070699).

Kinetic effects of mutations in residues involved in metal binding to the LIM-domain of a rat brain agmatinase-like protein. Benítez, J.R.[1], Vallejos, A.[1], Montes, P.[1], Cofré, J.[1], Romero, N.[1], Hidalgo, Á.[1], García, D.[1], Martínez, J.[2], Carvajal, N.[1], Uribe, E.A.[1]. Laboratorio de Enzimología, Departamento de Bioquímica y Biología Molecular, Facultad de Cs. Biológicas, Universidad de Concepción.[1], Laboratorio de Biofisica Molecular, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.[2].
Agmatine (decarboxylated arginine) has been associated to neurotransmitter, anticonvulsant, antineurotoxic and antidepressant actions in the brain. Agmatine levels would be, certainly, regulated by synthesis by arginine decarboxylase and hydrolysis by some agmatinase. However, whereas arginine decarboxylase has been well characterized, there has been difficulties even in detecting an active brain agmatinase. We have recently cloned and immunohistochemically detected a rat brain agmatinase-like protein (ALP), whose amino acid sequence greatly differs from other agmatinases and exhibits a C-terminal LIM-domain. The protein was detected in the hypothalamic region, hippocampal astrocytes and neurons. Since truncated species, lacking the LIM domain exhibits a 10-fold increased kcat, and a 3-fold decreased Km value, our proposal has been that the domain functions as an autoinhibitory entity in ALP. To further evaluate this, we have now cloned and expressed the isolated domain. The purified LIM-domain contained 2 Zn^{2+} ions, was a competitive inhibitor for the truncated, Zn^{2+}-free species (K_i of 0,2 uM), but had no effect on the wild-type enzyme. Considering the involvement of C823, C877 and H846 in zinc binding to the domain, as deduced from a modelled structure, the C823A, C877A, H846A mutants were generated. The k_{cat} was not altered by the His864-Ala mutation, whereas 10 and 2-fold increased values were exhibited by C823A and C877A, respectively. Results obtained reinforce our postulated autoinhibitory role for the LIM domain, which appears, therefore, as critical for regulation of agmatine concentrations in the brain. Grant Fondecyt 1120663, DIUC211.037.013-1.0.

Comparison and classification of DNA polymerase chains, domains and sub-domains based on multiple sources of structural and sequence information. Slater, A.W.[1,2], Cifuentes, J.J.[1,2], Melo, F.[1,2]. Molecular Bioinformatics Laboratory, Millennium Institute on Immunology and Immunotherapy[1], Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile[2].
DNA polymerases are a very ancient protein family responsible for the preservation of genetic information. They are very diverse in terms of sequence similarity and also very complex in terms of structure. For these reasons, DNA polymerases represent a challenging study case in bioinformatics. These are currently classified into 6 different families according to their sequence similarity. Little effort has been made in the comparison of these proteins in terms of their structures. Structural comparison can be particularly useful for detecting relationships in highly divergent protein families, where traditional sequence comparison methods cannot detect a clear similarity signal.We present a detailed comparison at the structural level of all representative family members of DNA polymerases currently available at the PDB. We have used a method based on multiple structural alignments generated by different software to perform a hierarchical classification of DNA polymerases at the chain, domain and sub-domain levels. Our results show that the current classification of DNA polymerases is only consistent at the chain-level. However, an important degree of structural diversity within a family and between families is still observed. We present some examples where the structural comparison suggests the splitting of two classes within a family and where two distinct sub-domains from different families are structurally related. We conclude that structural information is needed to reveal relationships that cannot be detected by sequence comparison and thus to improve the comparison and classification of highly divergent protein families. ACKNOWLEDGEMENTS:FONDECYT-1110400/ICM_N°_P09-016-F .

LjLTP10 gene coding for Lipid Transfer Protein is involved in aerial organ morphology in *Lotus japonicus* and have a role in cuticle formation and acclimation during drought stress. Tapia, G.[1], Alcorta, M.[1], Parra, C.[2], Morales, L.[2], Berbel, A.[3], Madueño, F.[3]. INIA-Quilamapu, Chillan, Chile[1], Universidad de Talca, Talca, Chile[2], IBMCP, Valencia, España[3]. Sponsored by Ruiz, S.
Non specific lipid transfer proteins (nsLTPs) are ubiquitous, small and basic proteins, that posses the ability to transfer lipid between membranes in vitro. In Arabidopsis they conformed a family of 49 members, and 156 in wheat. Several functions have been suggested for LTPs in different species, as signaling in pathogen defense, drought tolerance, stigma pollen adhesion, cell elongation or nodule formation.In *Lotus japonicus* genome we identified 25 sequences coding for LTPs, which were classified in seven groups. We filtered using microarray database for genes expressed in aerial organs of L. japonicus. We found four genes that were specifically expressed in shoots, leaves and stems, which were designed as LjLTP6, LjLTP8, LjLTP9 and LjLTP10. During drought stress, genes LjLTP6 and LjLTP10 were induced, the first, specifically in shoots and mature leaves, while the second in all aerial organs. LjLTP10 induction by drought stress was positively regulated by Methyl jasmonate but negatively by ABA, Ethylene and Hydrogen peroxide.Lotus japonicus RNAi mutants for LjLTP10 showed deficiency in leaf development with deformations, changes in dispositions and wide of leaflet, together with slow growth. Using in silico modeling and docking studies of LjLTP10 we identified main residues involved in ligand stability of palmitoyl CoA, the first candidate for bind to the protein. We suggest main role of these proteins associated with transport of precursors for cuticle formation in aerial organs, restructuring and drought stress tolerance of *Lotus japonicus*.Fondecyt Iniciación N°11090243.

Understanding non-trivial protein topologies using simplified molecular dynamics. Ramírez-Sarmiento, C.[1], Villalobos, P.[1], Baez, M.[2], Guixé, V.[1], Babul, J.[1]. Departamento de Biología, Facultad de Ciencias, Universidad de Chile.[1], Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.[2].
The increase of resolved structures deposited in the Protein Data Bank has allowed some intriguing non-trivial protein topologies to gradually populate this repository. The most remarkable examples are knotted proteins and domain-swapped dimers, which are challenging experimentally and theoretically and lead to difficult questions: How can a knotted protein reach the native state from a fully extended polypeptide chain? How can subunits exchange structural elements to form an intertwined dimer while keeping the same architecture observed for the monomer?To answer these questions, we employed the energy landscape theory and structure-based molecular dynamics to study the folding of DNA-binding proteins representing these topologies. Simulations of knotted protein VirC2 shows that the last step in its folding corresponds to threading the knot by either plugging or slipknotting. The occurrence of this intermediate state is more likely as the temperature is lowered to favor the native state. Simulations of FoxP2 and CSP from *B. caldolyticus* show that transitions between monomers and domain-swapped dimers are connected by the unfolded states and are concentration-dependent. The domain-swapped conformation matches the experimentally observed for oligomers, although the FoxP2 intertwined dimer is only transiently stable and quickly exchanges to another conformation where native contacts remain unaltered but dihedral angles are modified, suggesting that secondary structure rearrangements are important for the stability of the FoxP2 domain-swapped dimer. Consequently, these simplified models are successful in giving insights about the folding of proteins with complex topologies. Fondecyt 11110534 and 1090336.

Knotted and untied topologies of single-chain ARC repressor characterized by optical trap force spectroscopy. Bustamante, A.[1], Reyes, J.[1], Obando, P.[2], Guerra, D.[2], Wilson, C.A.[3,4], Bustamante, C.[3,4], Baez, M.[1]. Laboratorio de Bioquímica, Departamento de Bioquímica y Biología molecular. Facultad de Ciencias Químicas y Farmacéuticas. Universidad de Chile. [1], Laboratorio de Moléculas Individuales, Universidad Peruana Cayetano Heredia[2], Institute for Quantitative Biosciences (QB3), University of California, Berkeley, USA.[3], Jason L. Choy Laboratory of Single-Molecule Biophysics, University of California, Berkeley, USA[4]. Sponsored by Cabrera, R.
The Ribon-Helix-Helix family of transcription factors (RHH) presents a conserved structure created by two RHH motifs formed upon association of two identical polypeptide chain, (RHH_2). The bacteriophage P22 ARC repressor is a type RHH_2 homodimer, which can be converted into a single-chain monomer (mARC) adding a 15-residue glycine-rich linker to connect the C-terminus of the first RHH with the N-terminus of the second RHH motif. The folding mechanism and DNA binding of mARC remain unaltered with respect to the wild-type, dimeric protein. Interestingly, the 15-residue glycine-rich linker creates a loop able to knot the structure of mARC. Flipping movements of the glycine-rich linker around the protein can originate a knotted or untied topology of mARC. Using optical trap force spectroscopy, we explored the folding mechanism of mARC molecules as they were pulled from the C and N-terminal extremes attached to pair of DNA handles. Analysis of 500 unfolding events from eight individual fibers show two conformations for mARC characterized by contours lengths of 36 (4 fibers) and 43 nm (4 fibers). These values are expected for stretching a trefoil-knotted and unknotted polypeptide, respectively. Further calculations using non-equilibrium approximation indicate that the putative knotted conformation is stabilized by 6 kcal/mol relative to the unknotted, fully unfolded conformation. It is postulated that enhanced stability occurs upon constrain the unfolded state of mARC into a knotted topology. Fondecyt 11110534. Becario Conicyt 22121199.

GENE EXPRESSION I

Transcriptional regulation in the 4th dimension: unravelling circadian transcriptional networks in a model eukaryote. Montenegro-Montero, A.[1], Goity, A.[1], Weirauch, M.T.[2], Yang, A.[2], Hughes, T.R.[2], Larrondo, L.F.[1]. Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile[1], Banting and Best Department of Medical Research, Donnelly Centre for Cellular and Biomolecular Research, University of Toronto[2].

Transcription factors lie at the core of every gene regulatory network and their concerted and combinatorial interactions with *cis*-regulatory modules, contribute to the complex and precise temporal and spatial regulation of gene expression. Circadian clocks control the time-of-day-specific expression of hundreds of genes in different organisms, allowing for diverse cellular processes to take place at optimal times. The ascomycete *Neurospora crassa* has one of the best-understood circadian systems and a wealth of knowledge has accumulated on the molecular basis of its central oscillator. How the information is transmitted out of the oscillator to control overt rhythms however, is largely unknown. Our group is interested in characterizing regulatory circuits that allow the Neurospora clock to temporally control gene expression and the activity of its targets. We have taken a global approach for identifying transcriptional regulators displaying rhythmic expression profiles. By using a luciferase-based high-throughput screening system, we now show that the expression of several members of the eukaryotic-specific bZIP family is controlled by the circadian clock in Neurospora. To identify and characterize regulatory networks in which these and all Neurospora transcription factors participate, we are using double-stranded DNA microarrays containing all possible 10-base sequences to examine their binding specificities and in that way, predict possible targets on a genome-wide level, which we can then evaluate through genetic approaches. The combination of these methodologies allows for a circadian functional genomics strategy aimed at characterizing transcriptional networks in the Neurospora circadian system. FONDECYT-1090513.

The cytosolic H3K9me1 modification occurs during the synthesis of the histone. Alvarez, F.[1], Díaz Celis, C.[1], Ugalde, V.[1], Imhof, A.[2], Loyola, A.[1,3]. Fundación Ciencia & Vida, Santiago, Chile[1], Adolf-Butenandt-Institute, Ludwig-Maximilians-University, Munich, Germany [2], Universidad San Sebastián, Santiago, Chile[3].

The processing of histones H3 and H4 in the cytoplasm includes the translation of the mRNA by free ribosomes and the translocation to the nucleus of the newly synthesized proteins. Our group has recently described that this processing is carried out in a cascade of reactions that involves the correct folding of the histones H3 and H4 and the establishment of posttranslational modifications, associated to different chaperones. This cascade of maturation involves four different histone complexes. In contrast to the heavily modified nucleosomal histone H3, the cytosolic histones H3 have few modifications. They are acetylated at the residues lysine 14 and 18 (H3K14K18ac) and monomethylated at the lysine 9 (H3K9me1). Given that the modification H3K9me1 is observed in the first histone H3 complex, comprised by the newly synthesized histone H3 and the chaperone Hsc70, we hypothesized that this mark occurs during the histone H3 translation. To demonstrate this, we isolated ribosomes and investigate its association to SetDB1, the enzyme that monomethylates histone H3K9. We found that SetDB1 is bound to the ribosome. Moreover, we observed that the ribosome has H3K9 methyltransferase activity, which is lost when the cells are knocked down on SetDB1. Our results suggest that histone H3 posttranslational modification patterns start forming when histones are synthesized. Funded by FONDECYT 1120170, Basal Project PFB16.

Expression and biogenesis of mirrorRNAs in mammals. Munita, R.[1], Parada, G.[1], Mattick, J.[2], Gysling, K.[1]. Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Chile[1], Garvan Institute of Medical Research, Australia[2]. Sponsored by Canessa, P.

The advent of new technologies, has uncovered a high degree of complexity in the mammalian transcriptome. The existence of a special type of natural antisense transcripts (NATs), which are perfectly complementary to mature mRNAs over several exons, including splice sites, has been reported. We refer to these NATs as mirrorRNAs. Until now, no detailed analysis has been done on their abundance in the mammalian transcriptome and on their biogenesis. We propose two hypotheses to explain the biogenesis of mirrorRNAs. One possibility is that precursor NATs are directly spliced using the complementary sequences of the consensus signals. A second possibility involves the generation of mirrorRNAs by an RNA dependent RNA polymerase (RdRP) that uses mature mRNAs as template. We now report a systematic analysis that shows the presence of several mirrorRNAs in human ESTs, cDNAs and strand-specific RNA-Seq data. We have experimentally validated some of these mirrorRNAs. Further, we found that some mirrorRNAs are polyadenylated and have a 5' poly(U) tail. Our results using *in vivo* splicing assays on four cell lines, show that the mammalian splicing machinery is unable to functionally recognize the complementary sequences of the consensus signals. In conclusion, our results suggest that mirrorRNAs are the result of RdRP activity. Further investigation is necessary to determine the protein responsible for the RdRP activity in human cells.Funded by the grants ICMP10-063-F, FONDECYT 1110392 and Proyecto Conicyt Apoyo de Tesis Doctoral AT-24100163.

The interaction between the beneficial bacterium *Burkholderia phytofirmans* PSJN and *Arabidopsis thaliana* induces transcriptomic changes and phenotypical responses across the complete lyfe cycle of plants. Poupin, M.J.[1], Timmermann, T.[1], Vega, A.[2], González, B.[1]. Laboratorio de Bioingeniería, Facultad de Ingeniería y Ciencias, Universidad Adolfo Ibáñez, Santiago, Chile.[1], Departamento de Ciencias Vegetales, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile. Santiago, Chile[2].

Plant growth promoting rhizobacteria (PGPR) colonize the rhizosphere of many plant species conferring them positive effects such as increased plant growth and reduced susceptibility to diverse stresses. The mechanisms behind the growth promoting mediated by PGPR in plants are poorly understood. Using the interaction between the PGPR *Burkholderia phytofirmans* PsJN and *Arabidopsis thaliana* plants, as a model, three main questions were addressed in this study: 1) does strain PsJN affects late ontogeny of plants? 2) do the phenotypical changes observed in plants correlate with transcriptional changes? and 3) do bacteria actively regulate the phenotypic and transcriptional changes observed in plants? Regarding these questions we found that: 1) a unique inoculation during germination affected different life history traits across the whole plant development; 2) in a transcriptome analysis, 408 genes were detected with differential expression in PsJN-inoculated plants, some of these genes are involved in stress response and hormone pathways. Specifically, genes implicated in auxin and gibberellin pathways were induced, suggesting that these pathways could explain some of the observed phenotypes in colonized plants and 3) heat-killed inactivated bacteria induced a more severe transcriptional response in plants than undisturbed-PsJN. Nevertheless, these transcriptional changes were not able to induce phenotypical modifications in plants, suggesting that integrity or/and an active bacterial metabolism are required to induce plant growth promotion. These findings provide novel and interesting aspects of PGPR-plants interaction and open new venues to study these relevant biological interactions. Fondecyt-3100040, NM-PFG P/06-009.

Identification of a novel gene encoded in intron 5 of *RUNX1* gene. Hinojosa, M.[1], Gutiérrez, S.[1]. Laboratorio de Regulación Transcripcional y Leucemia. Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción[1].

The t(8:21) is one of the most frequent chromosomal translocation found in leukemia. To date all the break points mapped for this translocation, are located in intron 5 of *RUNX1* gene. Interestingly, this intron exhibits structural chromatin elements like Topoisomerase II and DNase I hypersensitive sites, which have been wildly associated with presence of transcriptional cis regulatory elements. Therefore, we hypothesize that regulatory elements are harbored in intron5 of the *RUNX1* gene. To identify regions of intron 5 enriched in epigenetics marks characteristic of regulatory elements, we performed bioinformatics analysis of ChIPseq data available from the ENCODE database. Our results shown that in hematopoietics cells there is one region highly enrich in K4H3me, K4H3me3 and K27H3ac that colocalizes with DNase I hypersensitive sites. These marks have been associated with promoter modules. Indeed, when we cloned this region in the pGL3 basic reporter vector, we found that it activates expression of the reporter gene luciferase in an orientation dependent manner. Using ETS databases we have assemble a putative messenger for this promoter. Moreover, by RT-PCR we have shown that this RNA is expressed both in Jurkat and HL60 cells. Taken together our results demonstrate the presence of a novel gene encoded in intron 5 of *RUNX1* that is expressed in hematopoietic cells.FONDECYT 1100670.

The HIV-1 internal ribosomal entry site is active in lymphocytes. Vallejos, M.[1], Pino, K.[1], López-Lastra, M.[1]. Laboratorio de Virología Molecular, Instituto Milenio de Inmunología e Inmunoterapia, Centro de Investigaciones Médicas, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile[1].

The full-length mRNA of the laboratory adapted pNL4.3 clone of the human immunodeficiency virus type 1 (HIV-1) harbors an internal ribosomal entry site (IRES) element within its 5' untranslated region (5'UTR). The viral IRES allows the recruitment of the hosts translational machinery to the viral mRNA. The molecular mechanisms governing IRES function are not fully understood. In a recent report (Vallejos et al. 2012) describe the presence of an IRES in the 5'UTR of the natural viral variants isolated from clinical samples. These newly isolated HIV-1 IRESes displayed higher activity when compared to the pNL4.3 clone. In order to extend these findings we sought to identified key domains within the 5'UTR region of the HIV-1 mRNA that drive IRES activity. For this a mutational analysis of the natural variant 2 (VAR2) was performed in the context of a bicistronic mRNA. This study revealed the importance of the PBS and DIS domains in the IRES activity. Up to now the HIV-1 IRES activity has been assesed in the context of HeLa cells, which is not a natural host for the HIV-1 infection. In this work the IRES activity has been tested in Jurkat cell an immortalized line of T lymphocyte cells. Results confirm that the IRES element within the 5'UTR of the HIV-1 full length mRNA is active in lymphocyte cells. Work supported by FONDECYT 1090318 and Proyecto P09/016-F Iniciativa Científica Milenio del Ministerio de Economía, Fomento y Turismo.

Establishment of a cell culture Hepatitis B Virus replication system. Muñoz, F.[1,2], Muñoz , E.[1,2], Hernández, S.[2], Venegas, M.[3], Brahm, J.[3], Gálvez, A.[4], Villanueva, R.[2], Loyola, A.[1]. Fundación Ciencia & Vida[1], Universidad Andrés Bello[2], Hospital Clínico Universidad de Chile[3], Universidad San Sebastián[4].

Persistent infection with Hepatitis B virus (HBV) is a major cause of liver disease and hepatocellular carcinoma. The HBV genome replicates its DNA in the nucleus of the infected hepatocytes via an intermediary known as covalently closed circular DNA (cccDNA). The cccDNA is the replicative intermediate responsible for the persistent infection of hepatocytes, and it serves as template for the transcription of all viral RNAs. Within the hepatocyte nucleus, the cccDNA is organized as a minichromosome, associated with cellular proteins such as histones, and the viral HBV core and HBx proteins. To study the replication cycle of the HBV, we generated a Chilean representative HBV molecular clone from a patient chronically infected with the locally prevalent HBV, genotype F, whose nucleotide sequence is fully known. With this clone, we performed transient transfections on human hepatocarcinoma Huh-7 cells, and analyzed the different replicative intermediates of HBV. Our results showed that we generated a culture system that recapitulates the HBV replication and infectious cycle. This model will allow us to study the replication of HBV DNA under different conditions, and isolate the viral cccDNA minichromosomes to analyze both its protein components as the epigenetic state of the viral chromatin. Funded by ANILLO ACT1119, FONDECYT 1120170, FONDECYT 1100200, Basal PFB16, USS005-2011-R.

RUNX1 protein auto-regulates its expression from P1 promoter. Martínez, M.[1], Trombly, D.[2], Stein, J.[2], Stein, G.[2], Gutiérrez, S.[1]. Laboratorio de Regulación Transcripcional y Leucemia, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción[1], Department of Cell Biology and Cancer Center, University of Massachusetts Medical School, Worcester, USA[2].

RUNX1 is a transcription factor essential for hematopoiesis process; it belongs to the family of runt related transcription factors (RUNX). The RUNX1 gene is located on large arm of chromosome 21 and its expression is controlled by two promoters, the distal (P1) and proximal (P2). Both promoters are separated by 160Kb and have different expression patterns, with P1 isoform been mainly expressed in T cells. Previously, we are been identified by *in silico* analysis, five putative RUNX binding sites in promoter P1 and 5'UTR region of RUNX1 gene, suggesting that RUNX1 protein may have an auto-regulatory role for its expression. To test this hypothesis initially we performed ChIP assays using anti RUNX1 antibody. Our results show that RUNX1 protein is bound to P1 promoter in the hematopoietic Jurkat cells. Next, to determine the effect of RUNX1 on its own transcription, we knock down RUNX1 protein using RNAi against RUNX1 mRNA and measured the levels of RUNX1 heterogeneous nuclear RNA (hnRNA) by RT-PCR. Both RNAi tested resulted in an almost complete absence of RUNX1 protein; interestingly in the RNAi treated cells we observed and increase in RUNX1-hnRNA levels. Taken together our results demonstrate that RUNX1 negatively regulates its expression from P1 promoter. FONDECYT1100670.

GENE EXPRESSION II

Epigenetic silencing of key bone phenotypic genes during neuronal development. Aguilar, R.[1,2], Bustos, F.J.[3], Henriquez, B.[2], Rojas, A.[1,2,4,5], van Zundert, B.[2], Montecino, M.[1,2]. FONDAP Center for Genome Regulation[1], Center for Biomedical Research, Universidad Andres Bello, Santiago[2], Universidad de Concepción, Concepción[3], Universidad de Chile, Santiago, Chile[4], Pontificia Universidad Javeriana, Colombia[5].

Runx2 and Osterix transcription factors are essential for bone development and osteoblastic differentiation as they regulate the expression of key bone lineage-specific genes. In neurons, the regulatory networks that may trigger transcription of osteoblast-specific genes are active, although are unable to promote their expression. We aim to evaluate the contribution of epigenetic mechanisms during silencing of osteoblast-specific genes in neuronal cells. Hippocampal tissue from rat embryos (E18) and postnatal animals (P10, P30, and adults) was obtained to perform Chromatin Immunoprecipitacion studies using antibodies directed against covalent histone modifications and chromatin modifying enzymes. It was found that Runx2 and Osterix promoter sequences exhibit enrichment of the repressive H3K27me3 epigenetic mark in all the stages analyzed. Additionally, we detected the presence of Polycomb-group proteins Ezh1 and Ezh2, binding in a developmental stage-dependent manner at Runx2 and Osterix promoters, correlating their expression pattern. We also found the heterochromatin-associated H3K9me3 epigenetic mark in the adult stage in tight correlation with decreased histone H3 acetylation and enrichment of Histone Deacetylases HDAC 1 and HDAC2. Finally, we found a strong DNA methylation pattern at bone-specific genes that are expressed at late stages during bone formation. Together, our results support a model where epigenetic post-translational modifications ensure the silencing of non-neuronal genes during neuronal differentiation. CONICYT 24120931, UNAB-DI-70-12/I, FONDAP 15090007, FONDECYT 1095075, FONDECYT 3110138, and FONDECYT 1101012.

Steamer is a retroelement present in the mollusk *Mya arenaria*. Arriagada, G.[1,2], Goff, S.P.[2]. Departamento de Ciencias Biológicas, Universidad Andres Bello[1], Department of Biochemistry and Molecular Biophysics, Columbia University, USA[2].

Haemic neoplasia (HN) is a proliferative cell disorder of the circulatory system of the soft shell clam Mya arenaria. The elucidation of the etiology of HN has been a key issue since the discovery of the disorder. The fact that the disease can be transmitted between individuals suggests the involvement of an infectious agent. The detection of reverse transcriptase (RT), the signature replication enzyme of retroviruses, is a strong indicator of retroviral presence. We have detected high levels of RT in hemolymph of diseased clams, and low background activity in controls. The identification of the source of RT activity in the hemolymph of HN clams will confirm or discard the hypothesis that a retrovirus is the etiological agent of the disorder. Here we present our efforts to identify the source of RT activity in the hemolymph of Mya arenaria.We extracted RNA from cell-free hemolymph of neoplastic animals, and used PCR amplification and 454 sequencing to obtain a database of cDNA sequences. Screening the database identified several cDNAs with similarity to known retroviruses. Using primers based on these sequences, we have cloned overlapping cDNA segments of a retroelement-related RNA from these RNAs. The clones contain a long open reading frame (ORF) encoding a predicted protein with protease, reverse transcriptase and RNaseH. We found that related DNAs are also present in the genome of normal clams, although in less copy number. Steamer mRNA levels in Mya arenaria hemocytes correlates with disease status.

Contribution of arginine methyltransferases PRMT1 and PRMT4 to the vitamin D3-mediated transcriptional control in osteoblasts. Moena, D.[1], Montecino, M.[1]. Center for Biomedical Research and FONDAP Center for Genome Regulation, Faculty of Biological Sciences and Faculty of Medicine, Universidad Andres Bello, Santiago, Chile.[1].

In bone cells, vitamin D regulation mainly occurs by modulation of gene transcription through the vitamin D receptor (VDR). Upon ligand binding, VDR suffers conformational changes on its C-terminal domain allowing interaction with co-activators of the DRIP/TRAP/Mediator complex or the p160/SRC family. In osteoblastic cells, these two types of co-activator complexes are recruited to specific target genes either in a cyclical, sequential and mutually exclusive manner [e.g. 24-hydroxylase (24(OH)ase)] or alternatively, through a gradual and preferential association of one of these type of co-activators [e.g. osteocalcin (OC)]. The p160/SRC family contains intrinsic histone acetyl transferase (HAT) activity and can interact with other HATs (p300/CBP, PCAF) and arginine methyltransferases (PRMT1/PRMT4), hence forming multiprotein complexes that include different histone modifying activities. Asymmetric dimethylation of R residues at histone tails is catalyzed by PRMT1/PRMT4 methylases and is associated with increased transcriptional activity. Here, we report the contribution of PRMT1 and PRMT4 during vitamin D-dependent transcriptional response. Using chromatin immunoprecipitation (ChIP) we establish binding of PRMT1 and PRMT4 to specific target gene promoters (24(OH)ase and OC) in osteoblastic cells following vitamin D incubation. We also find that both promoters exhibit different patterns of vitamin D-mediated histone R-methylation. Together these results support a model in which histone methyltransferases PRMT1 and PRMT4 are critical components during vitamin D-mediated transcriptional regulation in osteoblastic cells. CONICYT 24121258, FONDECYT 1095075, FONDAP 15090007.

The mitochondrial protein MEF25 is an essential and highly specific PPR factor for RNA editing in *Arabidopsis*. Arenas, A.M.[1], Moreno, S.[1], Gómez, M.I.[1], Takenaka, M.[2], Jordana, X.[1]. Departamento de Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile[1], Molekulare Botanik, Universität Ulm, Germany[2].

Plant mitochondrial gene expression is controlled at the post-transcriptional levels and RNA editing is one of the most enigmatic post-transcriptional processes occurring in the plant organelles. This site-specific conversion of cytidine to uridine nucleotides is found in almost all land chloroplast and mitochondria. A family of nucleus encoded factors, the Pentatricopeptide Repeat Proteins (PPR), present tandem arrays of motifs that bind RNA sequences specifically, and additional domains in the C-terminus that could be play an important role in editing. In this work, we have characterized one Arabidopsis PPR protein named MEF25 for Mitochondrial Editing Factor 25. We have analyzed the RNA editing status of 350 editing site in Arabidopsis mitochondrial transcripts from wild type and two homozygous mef25 mutants plants. We have shown that MEF25 is essential for RNA editing at only one editing site in the nad1 mitochondrial transcript (nad1-308), which encodes a respiratory complex I subunit. Northern blot analysis showed that the pattern of nad 1 transcrips is not affected by the lack of MEF25. Fusion of the N-terminal 144 aminoacids of MEF25 to GFP confirm a mitochondrial localization for this PPR protein. Finally, MEF25 promoter has been characterized by fusion to the GUS reporter gene. FONDECYT-1100601, Núcleo-Milenio-P10-062-F, ApoyoTesis-CONICYT-N?24100161.

Evaluating the role of the photoreceptor/GATA-type transcription factor White Collar-1 in the pathogenicity of *Botrytis cinerea*. **Hevia, M.A.**[1], Canessa, P.[1], Larrondo, L.F.[1]. Depto. Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.[1].

Botrytis cinerea is a necrotrophic ascomycete fungus that affects over 200 plant species. In Chile it produces significant damage in agribusiness infecting pre and post harvest. It has been documented that light modulate the interaction between a pathogen and a host and, although this has started to be explored in plant models, little is known about the effect of light on a fungal pathogen. In order to assess this, we have started phenotypic and transcriptional characterizations in response to light in Botrytis. WC-1 is a Zn finger GATA-family transcription factor, containing two putative transcriptional activation domains, a nuclear localization signal and two PAS domains: one is involved in protein-protein interactions and the LOV domain (light, oxygen, voltage) is critical for its blue photoreceptor activity and for sensing the redox potential of the cell. Together with WC-2, WC-1 forms the White Collar Complex, a heterodimer that controls the expression of several genes in response to light in *Neurospora crassa*. Creating a delta bcwc-1 strain we show that BcWC-1 mediates some, but not all, light responses. Also, this strain is more sensitive to oxidative stress in a light dosis-dependent manner. Using *Arabidopsis thaliana* as a host for infection assays under different light regimes, we have observed decreased pathogenicity when lights are on for the delta bcwc-1 strain. These results suggest the involvement of BcWC-1 in the Botrytis pathogenicity process. Fondecyt-postdoc 3110127, Fondecyt 1090513, ICGEB-CRP CHI09-02, Beca Apoyo tesis doctoral AT- 24121100, Conicyt.

Regulation of shoot meristem activity by the histone acetyltransferase GCN5 in *Arabidopsis thaliana*. **Aquea, F.**[1,2], Long, J.[1]. Plant Molecular and Cellular Biology Laboratory. The Salk Institute for Biological Studies, La Jolla, California, USA.[1], Departamento de Genética Molecular y Microbiología. Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.[2].

The role of chromatin remodelling in programming and maintaining specific gene expression profiles in meristems has been shown to be crucial in plant development. GCN5 (also known as HAG1) is a histone acetyltransferase that participates in regulating developmental gene expression in several species. In *Arabidopsis thaliana,* plants harboring loss-of-function alleles of *GCN5* show shorter roots and a variety of pleiotropic defects in the shoot. These include loss of apical dominance, overproliferation of young buds and the production of abnormal structures around the inflorescence meristem. To determine the roles for GCN5 in the regulation of the shoot meristem, we analyzed the genetic interaction of the mutant allele *hag1-3* with the master regulators of shoot development, the class III homeodomain leucine-zipper (HD-ZIPIII) genes. We established that *REVOLUTA (REV)* is necessary for the overproliferation of buds in *hag1-3* whereas mutants for *PHABULOSA (PHB), PHAVOLUTA (PHV)* and *CORONA (CNA)* enhance the same phenotype. This genetic evidence suggests that *GCN5* is an antagonist of *REV* and *GCN5, PHB, PHV* and *CNA* all act in the same genetic pathway. To gain insight about the molecular mechanism that is behind this genetic interaction, we are working on the identification and characterization of the transcription factors that are target of GCN5. Our work reveals a genetic framework that connects epigenetic modifications with the regulation of the shoot meristem. Acknowledgment: Postdoctoral fellowship STIPAS (Science, Technology, and Innovation Program for the Americas) and Programa Mecesup2.

Study of translation initiation mechanism of full-length mRNA of the human T-cell leukemia virus type 1 (HTLV-1). **Olivares, E.**[1], Rossi, F.[1], Pino, K.[1], Navarrete, C.[2], Huidobro-Toro , J.P.[2], López-Lastra, M.[1]. Laboratorio de Virología Molecular, Instituto Milenio de Inmunología e Inmunoterapia, Centro de investigaciones medicas, Escuela de Medicina, Pontificia Universidad Católica De Chile[1], Centro de Envejecimiento y Regeneración, CARE, Departamento de Fisiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile[2].

The full-length mRNA of human T-cell leukemia virus type 1 (HTLV-1), possess a 5'cap structure followed by a long structured 5'untranslated region (5'UTR). Translation of most eukaryotic mRNAs depends on the recognition of the 5'cap structure, however some cellular and viral mRNAs can initiate translation via internal ribosome entry sites (IRES). Studies have suggested that the 5'UTR of HTLV-1 harbors an IRES element. However, these observations remain highly controversial as other reports suggest that translation initiation is exclusively cap-dependent. These observations prompted us further evaluate if the 5'UTR of the full length HTLV-1 mRNA indeed harbored an IRES. To this end a series of bicistronic mRNA harboring the 5'UTR of HTLV-1 in the intercistronic spacer were constructed. In vitro, IRES activity was detected. Additionally both cistrons showed distinct responses to K+ and Mg2+ varying concentrations, suggesting that ribosomal recruitment is different for both. The presence of an IRES was further confirmed using *Xenopus laevis* oocytes system. Results showed that the IRES activity of the HTLV-1 5'UTR was higher than the previously reported HIV-1 IRES activity. Furthermore, using bicistronics mRNAs with nonfunctional 5'cap structures also show that expression of the second cistron was not to be due to formation of a chimeric IRES element in the intercistronic space. The results of this study suggest that the HTLV-1 full length mRNA can initiate translation by a cap-independent mechanism. FONDECYT 1090318 y P09/016-F Iniciativa Científica Milenio del Ministerio de Economía, Fomento y Turismo.

Identification of novel molecular factors affecting nitrogen use efficiency in *Arabidopsis thaliana*. **Araus C., V.**[1], Vidal, E.A.[1], Puelma, T.[1], Gutiérrez, R.A.[1]. Center for Genome Regulation. Millennium Nucleus Center for Plant Functional Genomics. Departamento de Genética Molecular y Microbiología. P. Universidad Católica de Chile.[1].

Nitrogen (N) is an essential macronutrient for plants and its availability is a key factor determining plant growth and productivity. To meet the increasing food demand, one of the main agricultural practices to increase yield is to use of N-fertilizers. However, their massive use is limited by their high cost and their important detrimental environmental impacts. A major challenge involves indentifying the key factors determining crop nitrogen use efficiency (NUE). Despite the importance of understanding these processes, little is known about the molecular mechanisms regulating NUE. Toward this goal, we used a bioinformatics tool to find genes involved in NUE in *A. thaliana*. The most connected gene in the search was a scaffold protein that acts as a transcriptional co-regulator in *A. thaliana,* NE1. We evaluated NUE in an overexpressor line of NE1 (35s::NE1) and we found a significant decrease in the NUE, specifically for N-limiting condition. We also evaluated the phenotype of 35s::NE1 and we found a decrease in primary root length and biomass also in N-limiting condition. We have evaluated the expression of other genes predicted with a functional connection with NE1 and we found that NE1 represses NTR2.1 and Glutamine synthetase 2. Thus, NE1 might control NUE by controlling nitrate uptake and availability in *A. thaliana*.Acknowledgment: Milenio-P10-062-F, Fondap-15090007, Fondecyt-1100698, Howard Hughes Medical Institute, Beca de Estudios de Doctorado CONICYT, Beca de apoyo a la tesis doctoral 24121609.

MOLECULAR BIOLOGY OF THE CELL II

Regulation of endoplasmic reticulum - mitochondria communication by protein kinase A in stressed HeLa cells. **Bravo, R.**[1], Rodríguez, A.E.[1], Quiroga, C.[1], Paredes, F.[1], Parra, V.[1], Quest, A.F.G.[1,2], Lavandero, S.[1,2,3]. Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/Faculty of Medicine, University of Chile[1], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile[2], Department of Internal Medicine, University of Texas Southwestern Medical Center, USA[3].
The endoplasmic reticulum (ER) is a crucial organelle for protein, lipid and calcium homeostasis. In response to perturbations, the ER triggers a variety of signaling pathways to communicate with different organelles. As part of this response, ER-mitochondria contacts increase to facilitate calcium transfer between both organelles and boost mitochondrial bioenergetics. mTOR is a key regulator of cellular metabolism; however, whether this protein participates In the control of interorganelle exchange is not known. We addressed this question in HeLa cells, a model where we previously evaluated mitochondria-ER interactions. Both rapamycin-mediated mTOR inhibition and tunicamycin-induced ER stress increased interorganelle proximity, as assessed by fluorescence confocal microscopy. Increased calcium transfer, mitochondrial network fusion and a higher rate of oxygen consumption paralleled these changes, suggesting an increase in mitochondrial bioenergetics. Addition of the PKA inhibitor H89 induced further increases in ER-mitochondrial proximity, suggesting an inhibitory role for PKA in the formation of interorganelle contacts. However, both mitochondrial fusion and augmented oxygen consumption were prevented by H89 treatment, suggesting that PKA, despite inhibiting ER-mitochondria communication, is important for maintaining mitochondrial bioenergetics. Our results suggest a dual role for PKA in the control of mitochondrial physiology: on the one hand, PKA reduces ER-mitochondria proximity, but on the other, enhances mitochondrial function. How PKA accomplishes both these effects requires further research. Supported by Anillo ACT1111 (AFGQ, SL), FONDECYT 1120212 (SL), CONICYT 24121339 (RB) and FONDECYT 3120220 (CQ). RB. AR and FP hold a CONICYT fellowship.

Long non-coding mitochondrial RNAs regulate Cyclin D1 and Survivin. Fitzpatrick, C.[1,2,3], Araya, M.[1,2], Briones, M.[1,2,3], Oliveira-Cruz, L.[1,2], Burzio, L.O.[1,2,3], Burzio, V.A.[1,2,3]. Fundación Ciencia para la Vida[1], Andes Biotechnologies S.A.[2], Facultad de Ciencias Biológicas, Universidad Andres Bello[3].
The novel family of non-coding mitochondrial RNAs (ncmtRNAs) displays a differential expression pattern between cancer and normal cells in human and mice. Normal proliferating cells express the sense (SncmtRNA) and antisense ncmtRNAs (ASncmtRNA). In contrast, tumor cells express the sense transcript and down-regulate de ASncmtRNAs. Treatment of melanoma cancer cell lines with oligonucleotides complementary to the ASncmtRNA induces inhibition of cell proliferation and massive and selective cell death by apoptosis. The viability of normal cells is unaffected by the same treatment. Besides apoptosis, knock-down of the ASncmtRNAs also induces strong down-regulation of Survivin, a member of the inhibitor of apoptosis (IAP) family which is up-regulated in all cancer cells. In summary, the treatment induces a combined effect on melanoma cell death: apoptosis and repression of Survivin, one of the most important anti-apoptotic proteins. The expression of Cyclin D1 is also markedly down-regulated and this effect could explain the Initial inhibition on cell proliferation, suggesting a functional role of these transcripts on the cell cycle. Other results indicate that down-regulation of these proteins is at the translational level. We hypothesized that the double-stranded region of the ASncmtRNAs could be processed by Dicer generating new microRNAs (Mito-miRs). Bioinformatic analyses showed that several candidate Mito-miRs could be generated by Dicer from the ASncmtRNAs, and some of them show the characteristic "seeding" sequence at the 5' end for interaction with Survivin or Cyclin D1 3' UTR mRNA (Supported by PFB-16 and Fondecyt 1110835 (CONICYT).

Participation of VCAM-1 and insulin in the ischemic cardiomyocyte protection. García, L.[1], Humeres , C.[1], Díaz, A.[1], Cordero, G.[1], González , V.[1], Corbalán , R.[2]. Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile[1], Facultad de Medicina, Universidad Católica de Chile[2].
Introduction and objective. Vascular cellular adhesion molecule-1 (VCAM-1) is a cell adhesion molecule of the immunoglobulin family involved in inflammatory processes. This protein has also non-immune functions because is essential for heart development and mice null for this protein are not viable. We have evidence that VCAM-1 is expressed in rat cardiomyocytes and insulin increases its levels. Insulin exerts cardioprotective effects by inhibiting apoptosis and stimulating cell growth. Here we described whether insulin and VCAM-1 are involved In protecting cardiomyocyte under ischemia. *Methodology*. Neonatal rat cardiomyocytes were stimulated with 10 and 100 nM insulin before or during simulated ischemia. At the end of 8 hours of ischemia cell viability by Trypan blue exclusion, subG1 apoptotic population by flow cytometry, LDH release, and VCAM-1 by inmunowestern blot were evaluated. Effect on viability of alfa4beta1 integrin (VCAM-1 receptor) stimulation on ischemia was assessed by TUNNEL. *Results*. Incubation with 100 nM insulin did not protect against cell death during ischemia. However, 10 nM insulin during ischemia produced a significant reduction of cell death involving necrosis and apoptosis. Insulin 10 nM increased VCAM-1 expression after ischemia. Alpha4beta4 integrin protected from cell death in the same extent than insulin. *Conclusion*. Insulin has cytoprotective effect and this effect is associated with increased VCAM-1. Integrin alpha4beta1 also protects from cell death. We planned to study cellular interactions in the process of protecting the viability of cardiomyocytes. *Fondecyt 1110346*.

Mitochondrial dysfunction and fragmentation induced by proinflammatory cytokines in 3T3-L1 adipocytes. Kuzmicic, J.[1,2], Hahn, W.[2], Burril, J.[2], Bernlohr, D.[2], Lavandero, S.[1,3,4]. Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/Faculty of Medicine[1], Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, USA[2], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile[3], Department of Internal Medicine, University of Texas Southwestern Medical Center, USA[4].
To assess the effects of proinflammatory cytokines on mitochondrial function, 3T3-L1 adipocytes were treated with TNF?, IL-6 or IL-1? (1 nM, 24 h) and evaluated for mitochondrial function as well as mRNA and protein expression. The treatment of 3T3-L1 adipocytes with inflammatory cytokines induced mitochondrial dysfunction as evidenced by a significant reduction ($p<0.05$, n=5) in maximal respiratory capacity (uncoupled respiration), increased proton leak, decreased ATP synthesis-driven respiration, respiratory control ratio and mitochondrial coupling efficiency. In addition, each of the cytokines reduced the NAD+/NADH ratio ($p< 0.01$, n=3). Each of the cytokines significantly reduced the mRNA expression PGC1? and TNF? also decreased the mRNA levels of NRF1 and COXIV, suggesting impairment in mitochondrial biogenesis. TNF? generated mitochondrial fragmentation ($p<0.05$, n=6), evidenced by an increase in mitochondrial number and decrease of mean mitochondrial volume. TNF? significantly reduced ($p<0.01$, n=4) the level of the mitochondrial fusion protein OPA1 without affecting the mitochondrial fission proteins DRP1 or FIS1. These results indicate that inflammatory cytokines induce mitochondrial dysfunction and changes in mitochondrial biogenesis/dynamics pathways, possibly contributing to the development of insulin resistance and type 2 diabetes mellitus. Supported by NIH DK084669 (DAB), Anillo ACT1111 and FONDECYT 1120212 (SL) and CONICYT 24110170 (JK). JK holds a CONICYT fellowship and a Fellowship from PABMB/ASBMB Promoting Research Opportunities for Latin American Biochemists.

Impaired mitochondrial Ca^{2+} uptake in pathological cardiomyocyte hypertrophy disrupts the proper insulin signaling. Gutiérrez, T.[1], Parra, V.[1], Troncoso, R.[1], Morales, P.E.[1], Contreras-Ferrat, A.[1,2], Vásquez-Trincado, C.[1], Lavandero, S.[1,2,3]. Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/Faculty of Medicine.[1], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile.[2], Department of Internal Medicine, University of Texas Southwestern Medical Center, USA.[3].

Cardiac hypertrophy is a physiopathological condition characterized by an increase in myocardium size and disruptions in energy metabolism, showing a reduction in fatty acid oxidation and insulin response. Our group has previously shown that Ca^{2+} release through IP_3 receptors, which are mainly localized in endoplasmic reticulum (ER) surface, plays a key role in insulin signaling. Additionally, mitochondria are important regulators of Ca^{2+} signals, acting as a buffer and uptaking Ca^{2+} after an increase in cytoplasmic levels. Here we evaluate the relationship between insulin signaling desensitization and Ca^{2+} signaling in hypertrophic neonatal cardiomyocytes. Insulin-induced cytoplasmic and mitochondrial Ca^{2+} signals were determined by confocal microscopy. The results show that the insulin-dependent increase in mitochondrial Ca^{2+} was significantly reduced in hypertrophic cardiomyocytes; however, the cytoplasmic Ca^{2+} increase remains unchanged. Similar results were obtained after stimulation with histamine, suggesting that this abnormal response is related with a reduced Ca^{2+} transfer between ER and mitochondria. In addition, we quantify the degree of ER and mitochondria colocalization, which showed a reduced coupling during hypertrophy. Interestingly, the blockade of Ca^{2+} entry to the mitochondria induces a reduction in insulin response similar to hypertrophy. In summary, these data suggest that insulin-induced mitochondrial Ca^{2+} signal is reduced in hypertrophy through a mechanism that involves a decrease in ER-mitochondria contacts, which may explain the reduced Ca^{2+} uptake and dysfunction experienced by this organelle. Supported by Anillo ACT1111 and FONDECYT 1120212 and 3110114. CVT and PEM hold a CONICYT fellowship.

Development and evaluation of oral vaccine prototypes against *Helicobacter pylori* in mice. Venegas, A.[1], Olmos-Mejías, M.[1], Moreno, F.[1], Melo, F.[1], Mosqueira-Dinamarca, M.[1], Berkowitz, L.[1], Chamorro, N.[1], Ávila, A.[1], Bajas, F.[1], Bruce, E.[1], Hernández, C.[1], Villagrán, A.[2], Harris, P.R.[2]. Laboratory of Microbial Pathogenesis and Vaccine Biotechnology, Department of Molecular Genetics and Microbiology, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Portugal 49, Santiago[1], Gastroenterology and Nutrition Unit, Division of Pediatrics, School of Medicine, Pontificia Universidad Católica de Chile, Marcoleta 391, Santiago[2].

We developed two prototypes of oral live vaccines based on an attenuated *Salmonella* strain and a lactic bacterium as carriers for expressing *H. pylori* antigens. We selected four antigens to be separately expressed by each prototype. Antigen genes were inserted into plasmids and transferred to bacteria by electroporation. Antigen expression was previously tested in vitro by Western blot using anti-His antibody to detect the antigens fused to a C-terminal His Tag. Previous studies showed that after single inoculation, recombinant vaccines strains persisted in mice intestine for at least 2-3 days without antibiotic selection. Also, a continuous bacterial culturing revealed that antigen expression last at least 72 hours. With this information we proposed a mouse vaccination protocol that included three oral immunizations ($1x10^8$ CFU each) every 48 hours followed by a similar booster 2 weeks later. Mice were challenged with *H. pylori* ($1x10^8$ CFU) at week 4 and after sacrifice (week 7-8), mice stomachs were removed for bacterial counting and q-PCR detection of *H. pylori* DNA. Also, blood samples were taken previous to immunization and then, at weeks 2, 4, and 7, to detect IgG response against the antigens. Results indicated that specific IgG responses were induced against the antigens and a reduction of *H. pylori* stomach colonization after single or mixed antigen immunizations was observed. Funded by Biomedicine Consortium BMRC-CTU 06-Area 5 and Fondecyt 1085232.

The Sall2 transcription factor is involved in p53-dependent apoptotic response to genotoxic stress. Escobar, D.[1], Sánchez, M.F.[1], Cerro, R.P.[1], Sanhueza, D.A.[1], Castro, A.F.[1], Pincheira, R.[1]. Laboratorio de Transducción de Señales y Cáncer. Departamento. de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.[1].

Sall2 is a member of the SALL/SPALT family of transcription factors, involved in normal development. Several studies suggest a relationship between Sall2 and cancer; however, the role of Sall2 in the disease is not well understood. Previously, we demonstrated that activated p53 tumor suppressor decreases both Sall2 protein and mRNA expression levels. We now investigated the functional relationship between Sall2 and p53. We isolated two types of mouse embryonic fibroblasts (MEFs); one derived from Sall2-deficient mice, and one derived from Sall2-deficient p53 knock-in mice. We investigated whether lack of Sall2 affects p53-dependent response induced by the chemotherapeutic drug, doxorubicin. Cellular apoptosis was examined through the activity of caspase-3 and caspase-7. Doxorubicin treatment induced apoptosis in Sall2+/+ MEFs, but this induction was barely detectable in Sall2-deficient cells. The latter result correlated with decreased both p53 activation and expression of some pro-apoptotic proteins. These findings reveal a new Sall2 function, and give insight into its relationship with p53 that could be relevant to cancer. FONDECYT 1110821.

Endocytosis regulation at transcriptional level in *Arabidopsis thaliana*: bZIP25 a novel functional transcription factor of endocytosis. Pizarro, L.[1], Osorio, C.[1], Norambuena, L.[1]. Laboratorio de Biología Molecular Vegetal. Departamento de Biología. Facultad de Ciencias. Universidad de Chile[1].

Endocytic pathway corresponds to cellular trafficking pathways involved in the internalization of plasma membrane and extracellular components to intracellular compartments. In plants and animals endocytosis participates in several processes such as signaling, extracellular nutrient uptake and cell polarity maintenance. To understand endocytosis functioning we are studying its transcriptional regulation components. We have found a novel trafficking regulator in *Arabidopsis thaliana* the transcription factor bZIP25. *bzip25-2* loss of function mutant shows accelerated endocytosis measured by the internalization of FM4-64 endocytic tracer. This result evidences a relationship between bZIP25 and endocytic route. To understand bZIP25 function we are using pharmacological tools to perturb trafficking pathways, and analyzing bZIP25 role in drug-response. We analyze the sensitivity of *bzip25-2* to BrefeldinA and Wortmannin, two endocytic pathways inhibitors, and Sortin2, an endocytic pathway inducer. This mutant shows a resistance to both inhibitors and hypersensitivity to the endocytic inducer. The resistance to inhibitors is consistent with constitutive accelerated endocytosis in *bzip25-2*; as well the hypersensitivity to the inducer is explained by the additive effect to its increased endocytosis. Gene transcript level analysis shows that under drugs treatment *bzip25-2* mutant has a diminished endocytic genes drug-response compare to wild-type plants, suggesting that bZIP25 is a component of endocytic pathways regulation in response to perturbing stimulus. Our result shows a novel negative regulator of endocytic pathway bZIP25 that is involved the response to endomembrane system perturbation in Arabidopsis. FONDECYT11080240. FONDECYT1120289. Conicyt-Chile PhD-Student Fellowship. Conicyt-Chile PhD-thesis Grant.

POSTER SESSIONS

1.Effect of phorbol 12-myristate 13-acetate on a nuclear cathepsin L variant and the activation of CDP/Cux transcription factor in colon cancer cells Caco-2. **Hermosilla, V.**[1], Iribarren, C.[1], Flaig, D.[1], Rivas, F.[1], Gutiérrez, S.[1], Morín, V.[1]. Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción[1].

Phorbol esters are diacylglycerol analogs that act as tumor promoters, being Phorbol 12-myristate 13-acetate (PMA) the most widely used. The effector of this compound is protein kinase C (PKC), a family of phospholipid-dependent serine-threonine kinases involved in several signaling transduction pathways. Derregulation of PKC may affect several downstream effectors such as Cathepsin L, a cysteine protease with a major role in protein turnover within the lysosomes. However, Cathepsin L also performs important functions in other cellular compartments, such as the cleavage and activation of CDP/Cux transcription factor within the nucleus during G1/S transition, allowing expression of genes involved in DNA synthesis. The effect of PMA on cell cycle progression from G1/S transition was studied in Caco-2cells through the incorporation of 5-bromo-2?-deoxyuridine. The expression of a nuclear Cathepsin L variant was determined through Western blot and RT-PCR; cellular location was evaluated by immunocytochemistry, whereas protease activity *in vitro* as well as *in vivo* was assessed by fluorogenic substrates cleavage and by CDP/Cux activation. With these techniques it has been demonstrated that PMA produces a delay in the exit from S phase. By treating cells with PMA for 4 hours from G1/S transition, a diminishing in the expression and activity of Cathepsin L at nuclear level was observed, producing a lower activation of CDP/Cux. These effects are reverted by coincubating with the PKC inhibitor chelerythrine, suggesting that they would be mediated, at least in part, by PKC. Grant: DIUC 212.037.016-1.0.

2.Expression and involvement of a cathepsin L variant in cell cycle progression of Caco-2 cells. **Rivas, F.**[1], Flaig, D.[1], Perez, V.[1], Hermosilla, V.[1], Leonardi, M.[1], Puchi, M.[1], Morin, V.[1]. Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción[1].

Cathepsin L is a cysteine protease often overexpressed in cancer cells. in our laboratory, a new nuclear Cathepsin L variant has been described in HeLa and Caco-2 cells. This 60 kDa variant has a differential localization through the cell cycle, displaying a nuclear location in S phase whereas in mitosis, it locates in the mitotic spindle where it colocalizes with alpha-tubulin. In the present work, we analyzed the expression and involvement of this new nuclear Cathepsin L variant in Caco-2 cell cycle, and evaluated the effect of its inhibition. We observed this new Cathepsin L variant displays a stable expression pattern through the cell cycle, and the treatment of cultured cells with Cathepsin L inhibitor I causes a decrease in its expression. We also observed that Cathepsin L inhibition results in a decrease of cell proliferation, loss of cell synchronicity and also alters subcellular localization of the new Cathepsin L variant during S phase and mitosis. Cathepsin L inhibition causes a delay in cell cycle progression, as observed in mitotic phase exit, also affecting mitosis marker H3 histone phosphorylation levels. As previously described, S phase entry also undergoes a delay due to Cathepsin L inhibition. Given these results, we suggest this new nuclear Cathepsin L variant as an essential factor in Caco-2 cell cycle progression, as well as an useful therapeutic target in human malignancies. FONDECYT 11070067/DIUC 212.037.016-1.0 .

3.Development of a lactate-detection assay for screening of anti-tumor compounds. **Venegas Faúndez, F.A.**[1], de Almeida Gonçalves, K.[2], Dias Gomes, S.M.[2]. Universidad Andrés Bello, Escuela de Bioquímica, Av. República 239, Santiago, Chile.[1], Biosciences National Laboratory (LNBio), Brazilian Center for Research in energy and materials (CNPEM)-Campinas SP, Brazil.[2].
Sponsored by Cabrera, R.

The aerobic metabolism of glucose, and high levels of glutaminolysis in cancer?s ceils supply for the high energetic and biosynthetic demands. A strong effect of the intensification of these pathways is the increase on the secretion of lactate by the tumor cells. The search of compounds capable of inhibiting enzymes of the glycolytic and glutaminolytic pathways is a recent new opportunity on the development of new cancer therapies, with the promise of high efficiency and range of action, since they are based on common elements of these type of cells: the metabolism reprogramming focused on promoting biosynthetic activity. The therapy with anti-glycolytic and anti-glutaminolytic compounds has the potential to stop cell proliferation, induce apoptosis and reduce the drug resistance of tumor cells.The goal of this project is to develop a biological assay aiming at the search for inhibitors of the glycolytic and glutaminolytic pathways. The bioassay is composed of the enzyme lactate dehydrogenase (LDH), which promotes the convertion of lactate to pyruvate, using NAD as a proton acceptor to produce NADH. The NADH is used to reduce the compound rezasurin releasing resorufin (a fluorescent compound) in the presence of the enzyme diaphorase. The developed assay is currently being used to detect differences in lactate levels secreted by four different tumor cells (two of breast cancer and two of prostate cancer), with and without treatment using one anti-glutaminolytic compound (2-deoxyglucose) and two inhibitors of the glutaminolytic pathway (BPTES and 986).

4.CRTC2 and BRG1 interaction is modulated by DNA damage in B cells. **Zambrano, A.**[1,2], Teitell, M.[2]. Instituto de Bioquimica y Microbiologia, Facultad de Ciencias, Universidad Austral de Chile. Chile.[1], Department of Pathology and Laboratory Medicine. University of California Los Angeles. USA.[2].
Sponsored by Concha, I.I.

Germinal Center (GC) microenvironment is the main source of memory B cells and plasma cells. During Class switch recombination, the activation-induced cytidine deaminase (AID) generates double strand breaks (DSBs), followed by repair of distal severed DNA ends. In this context, key components of the DNA damage response (DDR) are suppressed to allow GC B cell replication without causing senescence or apoptosis. Recently, it has been shown that DSBs leads to the phosphorylation and inactivation of CRTC2, a transcriptional co-activator for CREB. This inactivation shuts off a genetic program that control GC B cell proliferation. Interestingly, CRTC2 seems to directly target CREB-independent genes through interactions with other transcriptional regulators. We postulate that CRTC2 controls an alternative regulatory network through interactions with non-CREB transcription elements. Using CRTC2-IP followed by nLC-MSMS we determined an interaction between CRTC2 and BRG1, which is the central catalytic ATPase of the SWI/SNF chromatin-remodeling complex. Using Ramos B-cells we tested the CRTC2 association with BRG1 and demonstrated that CRTC2 binds BRG1 and forms a complex on CRTC2-target promoters. Additionally, we analyzed the CRTC2/Brg1 complex stability in cells undergoing DDR. Our results suggest that DNA damage, induced by etoposide, has an important role on CRTC2/BRG1 complex stability. Interestingly, DDR does not affect the occupancy of CRTC2-target promoters by BRG1. Founded by DID-UACh.

5.Identification of genes that encode for the enzyme lycopene b-cyclase in *Malus domestica* and its evaluation by heterologous expression in *Escherichia coli*. **Díaz, G.**[1], Cerda, A.[1], Stange, C.[1]. Laboratorio de Biología Molecular Vegetal, Facultad de Ciencias, Universidad de Chile[1].

Carotenoids are pigments found in chloroplasts and chromoplasts in plants. When accumulated, they give coloration to flowers, fruits and modified roots. They play an accessory role in the photosynthesis and exert also a photoprotective role in plants. b-carotene, in particular, works as an antioxidant and precursor of vitamin A when ingested by mammals, being essential for the human diet. One of the most studied enzymes of the carotenogenic pathway is lycopene b-cyclase (LCYB), catalyzing the formation of b-carotene from lycopene. On the other hand, *Malus domestica* (apple) is a good source of fiber and vitamins and Chile is the second largest exporter worldwide. However, it has been reported the presence of traces of b-carotene in its hypanthium (flesh). To determine the reason for the low accumulation of b-carotene in apples, we made a genomic search to identify homologous genes of carotenogenic enzymes. Using the GDR database, two lycopene b-cyclase candidates were found, *MdLCYB1* and *MdLCYB2*. Expression analysis performed through qRT-PCR, indicated that *MdLCYB1* expresses mainly in leaves; and *MdLCYB2*, in fruits. Both genes were cloned in expression vectors and heterologous complementation was made in a strain of *Escherichia coli* carrying the pathway genes with a deletional mutation for *LCYB*. The functional evaluation was performed by HPLC measurement to determine the amount and composition of carotenoids. These results point out these genes encode for functional LCYB enzymes, suggesting that apple has the ability to produce b-carotene *in vivo*. Funding: FONDEF D10I1022.

6.Rheb regulates p27KIP through activation of AMPK. **Campos, T.**[1], Palma, M.[1], Ziehe, J.[1], Pincheira, R.[1], Castro, A.F.[1]. Laboratorio de Transducción de Señales y Cáncer, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.[1].

Rheb is a small GTPase that couples growth factor signaling to activation of the mammalian Target of Rapamycin Complex 1 (mTORC1), thereby regulating key processes of cellular metabolism. The activity of Rheb/mTORC1 is controlled by the TSC2 tumor suppressor that inhibits Rheb by acting as a GTPase-activating protein (GAP). TSC2 deficiency causes pathology characterized by tumors in many organs. Also, it has been associated with elevated activity of AMPK, a kinase involved in sensing metabolic conditions, which in turn induces cytoplasmic localization of the cell-cycle inhibitor p27 (p27KIP). Since cytoplasmic p27 is associated with poor prognosis in cancer, we investigated molecular mechanisms controlling AMPK input on p27 function. We have previously found that Rheb, through an mTORC1-independent mechanism, activates AMPK and reduces p27 levels in TSC2-null cells. To address whether Rheb regulates p27 through AMPK, we now analyzed Rheb regulation of p27 mutants carrying mutations on residues that are essential for its regulation by AMPK. Our results indicated that Rheb regulates p27 through a mechanism partially dependent on the activation of AMPK. In addition, we analyzed the regulation of p27 by Rheb in several cancer cell lines. Interestingly, we found that Rheb was unable to regulate p27 in some of the cancer cells analyzed. Rheb`s failure to regulate p27 correlated with the presence of PI3K/Akt activating mutations, highlighting the importance of the genetic context on the molecular mechanism that drives the regulation of p27 in cancer cells. FONDECYT 1120923.

7.Determination of the interaction between non-coding mitochondrial RNAs and double-stranded-RNA binding proteins. **Briones, M.**[1,2,3], Oliveira-Cruz, L.[1,2], Fitzpatrick, C.[1,2,3], Burzio, L.O.[1,2,3,4], Burzio, V.[1,2,3]. Fundación Ciencia para la Vida[1], Andes Biotechnologies SA[2], Universidad Andrés Bello[3], GrupoBios SA[4].

Our group described a family of human non-coding mitochondrial RNAs (ncmtRNAs) consisting of Sense (SncmtRNA) and Antisense (ASncmtRNA) transcripts, which contain an inverted repeat attached to the 5?end of the sense or antisense 16S mitochondrial rRNA, respectively. As a consequence, these transcripts form stem-loop secondary structures. Although the function of these transcripts is unknown, there is a strong relationship between their expression and proliferative status, observed by in situ hybridization (ISH). Normal proliferative cells express S and ASncmtRNA and tumor cells express only the SncmtRNA transcript, allowing us to hypothesize that these transcripts may play a role in proliferation and cancer. By analyzing the double-stranded RNA region of these transcripts, it is interesting to consider an interaction with proteins that possess dsRNA binding domains (DRBPs). DRBPs have specificity for stem-loop structures of many RNAs, in which we find different families of proteins involved in diverse cellular mechanisms. One candidate group of DRBPs that could interact with the ncmtRNAs are proteins involved in microRNAs (miRNA) synthesis such as Dicer and Drosha, since by bioinformatic analysis, we observe that there are putative microRNAs originated from ASncmtRNA with complementarity to the 3'UTR of cyclinD1. In this work, using RNA immunoprecipitation assays in SKMEL-2 melanoma cells, we observed an interaction between ncmtRNAs and Dicer, confirmed by FISH and Immunocytochemistry. This interaction indicates that the ncmtRNAs may be involved in miRNA generation, which could shed light on the function(s) of these transcripts. FONDECYT 1110835.

8.Hyperosmotic stress triggers cytoprotective autophagy in HeLa cells. **Oyarzún, A.P.**[1], Troncoso, R.[1], Rodríguez, A.E.[1], Paredes, F.[1], Gatica, D.[1], Bravo, R.[1], Vasquez-Trincado, C.[1], Quiroga, C.[1], Criollo, A.[2], Lavandero, S.[1,2,3]. Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/Faculty of Medicine, University of Chile[1], Department of Internal Medicine, University of Texas Southwestern Medical Center, USA[2], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile[3].

Hyperosmotic stress (HS) stimulates apoptosis in HeLa cells. HS also activate survival signaling, so the balance between death and survival pathways will determine the cell fate. Autophagy is a well-characterized survival response. This work is focused on the cytoprotective role of autophagy in HS-exposed cells. HS was induced by sorbitol and autophagy was studied by dots formation in U2OS-GFP-LC3 cells and LC3-I processing and p62 levels by Westernblot (WB). Phosphorylation of AMPK and mTOR, and levels of Beclin-1, ATG5 and caspase-3 were also assessed by WB. LC3 processing was investigated in ATG5-KO or ATG7-KO MEF cells. Cell viability was determined by mitochondrial membrane potential (ψmt) using TMRM or DiOC/PI staining. The protective role of autophagy was evaluated using beclin-1 and ATG5 siRNAs. The role of caspases on autophagy induction was assessed in Z-VAD-treated cells. Our data show that HS induces the redistribution of GFP-LC3, LC3-I processing, autophagic flux and decreased p62 levels. Also, HS stimulates AMPK phosphorylation and mTOR dephosphorylation. Sorbitol induced a drop on ψmt. At later times, HS activates caspase-3 and promotes Beclin-1, ATG5 and AMPK cleavage leading to cell death. Finally, autophagy was increased in Z-VAD treated cells. Altogether these results suggest that HS induces a cytoprotective autophagic response, which is diminished through caspase-3 activation and cleavage of Beclin-1, ATG5 and AMPK to induce cell death. RAE, PF, BR, VC hold PhD fellowships from CONICYT, Chile. ANILLO ACT1111. FONDECYT 1120212, 3110114 and 3120220 supported this work.

9.Characterization of *Neisseria gonorrhoeae* infection in human antigen presenting cells. **Villegas-Valdés, B.**[1,2], Tempio, F.[1], Reyes-Cerpa, S.[2], Imarai, M.[2], Acuña-Castillo, C.[2], Escobar, A.[1]. Facultad de Odontología-Universidad de Chile[1], Centro de Biotecnología Acuícola-Universidad de Santiago de Chile[2].
Introduction: *Neisseria gonorrhoeae* (Ngo) is the causal agent of the sexually transmitted disease gonorrhea. Results from our laboratory indicated that Ngo affects the expression of molecules involved in antigen presentation in murine antigen presenting cells (APC). The aim of this study is to compare the effects of infection with Ngo in murine APC versus human APC.**Material and Methods:** human dendritic cells (DCs) and a monocytic human cell line (THP-1) were infected with Ngo expressing GFP at different multiplicities of infection. Binding of bacteria to the APC and subsequent phagocytosis were evaluated by confocal microscopy after 6, 12 and 24 hours. Also, we analyzed the expression of molecular markers involved in antigen presentation by flow cytometry after 24 hours of infection.**Results:** Confocal microscopy analysis after 6 hours of Ngo infection indicated that the bacteria had been binded and internalized by both DCs and THP-1. Moreover, the expression of molecular markers of maturation as CD86 and MHC II is not affected in DCs. Also we detected by flow cytometry the activation of Th17 response. In the future, we will evaluate the mechanisms and effects involved in Ngo infection. **Discussion:** It is critical to understand the effect of Ngo on APC because these cells initiate the immune response against this bacterium. Comparison of results observed in the murine and human cells is helpful to understand and design therapeutic strategies to battle Ngo infection. **Acknowledgments DICYT021043IB, FONDECYT 11110304**.

10.Role of Pannexin-1 in the depletion of regulatory T cells. **López, X.**[1], Shoji, K.F.[2], Sáez, P.J.[2], Sáez, J.C.[2], Imarai, M.[3], Acuña-Castillo, C.[1]. Centro de Biotecnología Acuícola-Universidad de Santiago de Chile[1], Departamento de Fisiología-Pontificia Universidad Católica de Chile[2], Centro de Biotecnología Acuícola-Universidad de Santiago de Chile[3].
Regulatory T-lymphocytes (Tregs) are involved in producing and maintaining immune tolerance. We reported that polymyxin B (PMB), a positive modulator of the ionotropic purine ligand gated P2X7-receptor (P2X7R), depletes selectively Tregs from spleen population in a mechanism not related to P2X7R activation. Furthermore, purine-mediated apoptosis has been associated to P2X7R activation and to pannexin-1 (Panx1) hemichannel (HC) activation. In view to new reports our idea was to determine whether depletion of Tregs caused by PMB is associated to Panx1 HC activation. We studied the effect of ATP and PMB in lymphocytes from C57BL/6 and Panx1-/- mice in vitro and in vivo. Also, the effect of these agents was studied in the presence and absence of 10Panx (a Panx1 HC mimetic inhibitory peptide) and carbenoxolone (CBX) (blockers of Panx1 HCs) in vitro. Tregs from C57BL/6 mice were sensitive to PMB and ATP in a concentration-dependent manner. In contrast, Panx1-/- Tregs were completely insensitive to PMB and sensitive only to the maximum ATP concentration. Preliminary in vitro experiments showed that ^{10}Panx and CBX prevented the PMB-induced depletion of Tregs. Further experiments are needed to confirm this effect and also the effect of ATP and PMB in vivo. These findings suggest that PMB-induced depletion of Tregs is a Panx1-dependent process. Funded by FONDECYT 1110734, 11070177 and Anillo ACT-71.

11.PMB and ATP improves antitumoral response in mice. **Mena, J.**[1], Neira, T.[1], Capelli, C.[1], Montoya, M.[1], Escobar, A.[2], Mateluna, C.[1], Acuña-Castillo, C.[1], Imarai, M.[1], López, X.[1]. Centro de Biotecnologia Acuícola-Universidad de Santiago de Chile[1], Facultad de Odontologia-Universidad de Chile[2].
Regulatory T-lymphocytes (Tregs) are involved in producing and maintaining immune tolerance, and are increased under pathological conditions as cancer, this increase is related to bad prognosis. We previously determined that polymyxin B (PMB) plus ATP induce depletion of Treg in a P2X7 receptor (P2X7R) and pannexin mediated mechanism. In order to determine whether this effect correlates with induction of antitumoral immune response, we evaluated the effect of this treatment on B16 models of tumoral growth.C57BL/6 mice were intraperitoneally injected with PMB plus ATP, and Treg population was evaluated by flow cytometry. As expected, PMB plus ATP induced a decrease of spleen CD4+ CD25+ Treg cells 1 to 3 days after challenge, this mechanism was dose dependent. A slight decrease in conventional T cells was detected from day 3. Once determined that both drugs had a functional effect, we evaluated whether combined treatments could improve antitumoral activity. Thus, C57BL/6 mice were immunized 3 times in three weeks with B16 apoptotic bodies (AB) alone, plus ATP, plus PMB or both. After immunizations, animals were injected with B16 melanoma cells and tumor growth was evaluated daily. Twenty four days post treatment, all mice of non immunized group had developed tumors, while immunized groups with AB, AB+PMB, AB+PMB+ATP, showed 26%, 37% and 50%, respectively, of tumor free mice until 70 days post-treatment. In conclusion, our combined treatment improved the antitumoral effects. Proyecto FONDECYT 1110734, FONDEF VIU 110049.

12.Identification of a novel Gαs phosphorylation site. **Beyer, A.**[1], Pastén, P.[1], Torrejón, M.[1], Olate, J.[1], Hinrichs, M.V.[1]. Laboratorio de Genética Molecular, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Chile.[1].
In the canonical G protein-signaling pathway, heterotrimeric G proteins are activated by agonist-bound heptahelical receptors (GPCR), which promote the exchange of GDP for GTP on Gα subunits and subsequently their dissociation from Gβγ dimers. Recently, new receptor-independent GEFs (guanine nucleotide exchange factors) have been discovered, which are also participating in G protein activation. One of these new GEFs is Ric-8, a protein isolated in a two-hybrid screening as an interacting partner of Gα subunits. In mammals, two different orthologs have been reported, Ric-8A and Ric-8B, which possess the ability to interact with different Gα subunits. Ric-8A has been shown to interact with Gαi, Gαo and Gαq and Ric-8B with Gαs and Gαq. In previous studies we demonstrated that Ric-8B is translocated to the plasma membrane in response to isoproterenol, a β-adrenergic receptor agonist that activates Gαs. In order to gain some insights into the mechanism involved in Ric-8B translocation, we analyzed if Gαs activation induces any Ric-8B posttranslational modification. Indeed, through Western blot analysis, using an anti phospho-serine/threonine/tyrosine antibody, we demonstrated that Ric-8B was phosphorylated, when cells were incubated with isoproterenol. Interestingly, we also identified in Ric-8B immunoprecipitates, obtained from cell lysates treated under the same conditions, Gαs that was also phosphorylated. Through bioinformatic analysis, we identified serine 272 as the most likely PKA phosphorylation site. In order to study the physiological effects of this modification, we constructed and expressed the Gαs S272A mutant. Acknowlegments: FONDECYT GRANT 1090150.

13.Design and functional analysis of vectors to increase the carotenoid content in fruits. Peirano, C.[1], Stange, C.[1]. Laboratorio de Biología Molecular Vegetal, Facultad de Ciencias, Universidad de Chile[1].

Carotenoids are isoprenoid compounds with high antioxidant properties, synthesized in plastids of photosynthetic organisms like plants, some bacteria and fungus. Between them, B-carotene is an essential component in the human diet, because it is the main precursor of Vitamin A (retinol), which deficiency leads to blindness. Due to their benefits on human health, we proposed to develop a new apple variety, able to synthesize pro-vitamin A. In this work we used bioinformatics tools to optimize the phytoene synthase 2 (*PSY2*) gene of *Daucus carota*, enzyme that catalyzes the first and key step in the B-carotene pathway, and the downstream phytoene desaturase (crtI) gene of *Xanthophyllomyces dendrorhous*, fused to a chloroplast transit peptide (pt). The expression was directed by the fruit-specific promoter of tomato polygalacturonase gene (pPG). These sequences were used to synthesized the cassettes pPG::*PSY2*::nos and pPG::pt::crtI::nos, which were utilized to generate two self-made binary vectors that were transiently transformed in tomatoes and apple fruits by Agroinfiltration. Through RT-PCR analysis of these tissues, we successfully proved the transcriptional functionality of the cassettes. Additionally, by *PSY2* and crtI GFP C-terminal fusions we were able to check the chloroplast localization of both proteins in tomato. These results demonstrate the functionality of the cassettes and strongly suggest the use of these freedom to operate vectors to raise the carotenoid content in fruits, through plant metabolic engineering. Acknowledgements: To Fondef D10I1022 and Victor Cifuentes for kindly contribute with the *X.dendrorhous* crtI sequence.

14.Alteration of tumorigenic properties by knockdown of the antisense noncoding mitochondrial RNA in murine melanoma. Lobos-González, L.[1], Silva, V.[1], Fitzpatrick, C.[1], Bendek, M.[2], Silva, V.[3], Burzio, L.O.[4], Burzio, V.[1]. Andes Biotechnologies[1], Universidad Andres Bello[2], Universidad Andres Bello, Viña del Mar[3], Andes Biotechnologies, Fundacion Ciencias Para La Vida[4]. Sponsored by Burzio, V.

We described a family of noncoding mitochondrial RNAs (ncmtRNAs), containing sense (SncmtRNA) and antisense (ASncmtRNA) members. All proliferating cells express the SncmtRNA, but the ASncmtRNA is expressed only in normal proliferating cells and is down-regulated in tumor cells. Knock-down of the ASncmtRNA in cultured cells induces massive tumor cell death, leaving normal cells unaffected. The murine melanoma cell lines B16F0, B16F1 and B16F10 (increasingly metastatic) were treated in vitro with control (ASO-C) and therapeutic (1560S) ASO, respectively; treatment with 1560S caused massive cell death, diminished proliferative capacity and practically abolished anchorage-independent growth. These cellular alterations were accompanied, at the molecular level, by down-regulation of survivin and cyclin D1 proteins. Additonally, B16F10 cells treated ex vivo with therapeutic ASO were unable to induce formation of subcutaneous tumors in C57BL6/J syngeneic mice. On the other hand, the same cell line, when treated with the therapeutic ASO, diminished its wound-healing and transmigrational capacity. In conclusion, treatment with the ASO against the ASncmtRNA diminishes drastically the tumorigenic and invasive capacity of murine melanoma cells, supporting the use of this approach for the development of melanoma therapy.Supported by FONDECYT 1110835 (VB) and ANDES BIOTECHNOLOGIES.

15.Mitochondrial dysfunction in NK cells from elderly donors. Córdova, A.[1], Jara, C.[1], Martín, A.[1,2], Mejías, S.[1], Acuña-Castillo, C.[1], Miranda, D.[2], Montoya, M.[1]. Departamento de Biología. Facultad de Química y Biología. Universidad de Santiago de Chile[1], Laboratorio Inmunobioquimica. Facultad de Ciencias Químicas y Farmacéuticas. Universidad de Chile.[2].

Human NK cells contribute to host antimicrobial and antitumor defense reactions. These cells are able to spontaneously lyse target cells and produce an important amount of cytokines. IL-2 activates NK cells, promoting their migration within target tissues, increasing the secretion of cytokines and chemokines and inducing a strong cytolytic activity. However, during aging, NK cells show a decreased function and poorly respond to IL-2 activation. Although a large amount of research has been carried out about mitochondrial dysfunction in aging, nothing has been reported in literature about mitochondrial dynamics in old NK cells. In this respect, we postulated that NK cells from older adults failed to increase mitochondrial mass or mitochondrial membrane potential when stimulated with IL-2. NK cells were purified from peripheral blood mononuclear cell from healthy young or elderly donors, cultured with rhIL-2 and further analyzed for mitochondrial mass (MitoTracker? Green) and mitochondrial membrane potential (JC1). Our results showed that incubation with 2000 UI/mL rhIL-2 for 72h induce an increase in mitochondrial mass and in mitochondrial membrane potential compared to the control cells cultured with 50 IU/mL rhIL-2. Interestingly, purified NK cell from elderly donors did not show a significant increase in the analyzed parameters. Mitochondrial dysfunction has been related to different patophysiological conditions in aging. For the first time we described mitochondrial dysfunction related NK activation in aging which may be related to immunosenescence. Financial support: Fondecyt 11110401, Proyecto Bicentenario PDA-20.

16.Lactate effect on mitochondria and cellular oxidative status in B16 and MDA-MB231. Ahumada, V.[1], Ureta, A.[1], Ibañez, J.[1], Acuña-Castillo, C.[1], Miranda, D.[2], Montoya, M.[1]. Departamento de Biología. Facultad de Química y Biología. Universidad de Santiago de Chile[1], Laboratorio Inmunobioquimica. Facultad de Ciencias Químicas y Farmacéuticas. Universidad de Chile[2].

Increased lactate formation has been correlated with poor prognosis in cancer. Although, a high glycolysis rate has been associated with cancer formation, today is accepted that mitochondrial metabolism is essential for cancer cell maintenance and disease progression. Moreover, mitochondrial ROS production was related to metastatic capacity in cancer cells. On the other hand, we previously showed that mitochondria of cancer cell express proteins necessary for lactate oxidation, suggesting that lactate can be metabolized in this organelle. We postulate that lactate also can modulate metabolism in the highly metastatic B16 and MDA-MB231 cells, decreasing glucose uptake, increasing mitochondrial mass and/or membrane potential and affecting the oxidative cell status. In MDA-MB-231, incubation with high concentration of lactate for 96h decreased glucose uptake and induced an increased on mitochondrial mass, also an increase in ROS generation with an increase in the antioxidant capacity (GSH/GSSG) was detected. Although, B16 cells also show a decrease in glucose uptake and an increase in mitochondrial potential, did not shown any change in mitochondrial mass. More interestingly, B16 increase ROS formation with a decrease in GSH/GSSG ratio. The results show that high lactate concentration induces important changes in tumor cells metabolism and oxidative status. We can speculate that these changes can be related to tumor progression. Financial support: Proyecto Bicentenario PDA-20, Fondecyt 11110401.

17.Vitellogenin in male Chilean flounder *Paralalichthys adspersus*. Endocrine disruption biomarker. Morin, V.[1], Bustamente, S.[1], Bustos, P.[2], Puchi, M.[3], Romo, X.[3], Leonardi, M.[3]. Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción[1], Departamento de Bioquímica clínica, Facultad de Farmacia, Universidad de Concepción[2], Departamento de Ciencias Biológicas, Facultad de Ciencias Biológicas, Universidad Andrés Bello[3].

Recent studies performed in flounders captured from different sites of the coast of Biobío region, area under the influence of industrial and domestic discharges, showed:, high prevalence of plasmatic vitellogenin in male flounders and spermatic cell changes. These results demonstrated the presence of xeno-estrogenic pollutants in the coastal area. The vitellogenin in plasma has proved to be a simple and sensitive biomarker for assessing exposure of fish to environmental estrogens. Due to the lack of information about vitellogenin in marine organisms, we have induced, purified, and characterized this protein in blood plasma of males of the Chilean flounder Paralichthys adspersus, the main sentinel specie used in studies of marine monitoring environmental. These studies have allowed the production of polyclonal antibodies to quantify vitellogenin in blood of flounders P. adspersus. These studies have shown high levels of plasmatic vitellogenin in male caught in front of Itata River and Concepción Bay. The vitellogenin gene transcription quantified in livers of adult males by RT-PCR, using RNA obtained of male liver from Chilean flounders P. adspersus, corroborates the results obtained previously. The results suggest that estrogenic endocrine-disruption compounds are introduced into the marine environment, negatively affecting the fish studied. The relevance of this report is discussed in relation to estrogenic compounds introduced by industrial and municipal wastewater effluents.

19.*Arabidopsis thaliana* ion transport related gene 7: Functional analysis in ionic stress. Figueroa, A.I.[1], Urbina, D.C.[1], Norambuena, L.[1]. Laboratorio de Biología Molecular Vegetal, Departamento de Biología, Universidad de Chile[1].

In *Arabidopsis thaliana* we identified a novel gene product as putatively involved in ionic stress that we named ITRG7 (**I**on **T**ransport **R**elated **G**ene 7). ITRG7 is a transmembrane protein and has been classified as member of the plant drug and metabolite exporter family protein, however its function has not been described. Expression qRT analysis show that ITRG7 expression decrease in wild type plants treated with different types of ionic stress, indicating that under these conditions ITRG7 could have a rol. We have decided to aboard the characterization of ITRG7 by two pathways: reverse genetics and heterologous expression in yeast. If ITRG7 is involved in ionic stress response, a loss of function mutant could have phenotypic differences in the response pattern to this kind of stress. An *Arabidopsis* insertional mutant homozygous line has lower ITRG7 transcript level than wild type plants. This mutant has not difference in germination under media containing different ions. This could be explained by gene redundancy, nevertheless we are evaluating other phenotypes. The heterologous expression in yeast has shown that overexpression of ITRG7 leads to an increase of sensitivity to metal and saline stress. This increment of sensitivity is to a selected set of metals suggesting specificity in ITRG7 mechanism. Our results strongly suggest that ITRG7 participates in ionic stress response. This work was supported by Fondecyt 1120289 & 11080240.

18.*In silico* analysis of tetrahydrohyperforin suggests a potential pharmacophore for the activation of TRPC6 channels. Schüller, A.[1,2], Montecinos-Oliva, C.[3], Parodi, J.[3], Inestrosa, N.C.[3]. Molecular Bioinformatics Laboratory, Millennium Institute on Immunology and Immunotherapy[1], Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile[2], Centro de Envejecimiento y Regeneración (CARE), Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile[3]. Sponsored by Melo, F..

Tetrahydrohyperforin (IDN5706) is a semi-synthetic compound derived from hyperforin, the main active principle of St. John's wort, and has numerous beneficial effects in an Alzheimer's disease mouse model. However, the drug's mechanism of action is still unknown. We analyzed the field excitatory postsynaptic potential and long-term potentiation induced in mouse hippocampal slices and observed that IDN5706 and 1-oleyl-2-acetyl-sn-glycerol (OAG), an activator of canonical transient receptor potential channels (TRPC3/6/7), triggered similar effects, while the TRPC channel blocker SKF96365 inhibited the effects. IDN5706 further protected against neurotoxicity induced by amyloid-beta oligomers, the causative agent of Alzheimer's disease, and performance in the Morris water maze was increased by treatment with IDN5706 and reduced by co-treatment with SKF96365. This suggests that IDN5706 acts through activation of TRPC3/6/7 channels. We then analyzed IDN5706 and hyperforin, a known TRPC6 activator, by molecular docking and predicted a similar binding mode involving a conserved three-residue hydrogen bonding pattern. A similar result was obtained with the ligand-based method of pharmacophore alignment. The chemical structures of four potential TRPC6 activators (IDN5706, hyperforin, OAG, and the synthetic compound Hyp9) aligned well and shared a common potential pharmacophore of two hydrogen bond acceptors and one donor. We propose that the effect of IDN5706 is mediated through activation of the TRPC6 channel. Unveiling the action mechanism of IDN5706 is a necessary step towards the use of the drug in Alzheimer's disease. **Acknowledgments:** FONDECYT 3110009 to A.S., FONDEF D07I1052, FONDECYT 1120156, CONICYT-PFB12/2007 to N.C.I.

20.Inhibition of MCT1 through an adenoviral transduction of MCT1 shRNA induces a compensatory effect. Carril-Pardo, C.[1], Elizondo-Vega, R.[1], Cortés-Campos , C.[1], Nualart, F.[2], Uribe, A.[3], García-Robles, M.A.[1]. Laboratorio de Biología Celular, Departamento de Biología Celular, Facultad de Ciencias Biológicas, Universidad de Concepción[1], Laboratorio de Neurobiología y Células madres, Departamento de Biología Celular, Facultad de Ciencias Biológicas, Universidad de Concepción[2], laboratorio de Enzimología, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción[3].

It has been proposed that the brain glucosensing mechanism in the arcuate nucleus is carried out through a neuron-glia interaction mediated by lactate. Hypothalamic glial cells have a high capacity to incorporate glucose and release lactate, and both the monocarboxylate transporters, MCT1 and MCT4, are localized to tanycyte processes that contact arcuate nucleus neurons. To assess the relative contribution of MCT1 and MCT4 in the brain glucosensing mechanism, an adenovirus expressing shRNA that specifically inhibits MCT1 and also carries the EGFP reporter gene (AdshMCT1) was generated. Also, an adenovirus that inhibits expression of β-galactosidase and carries the same reporter gene was used as a control. shRNA-mediated inhibition of MCT1 expression was evaluated in primary cultures of tanycytes. The cell transduction efficiency over time was detected by immunofluorescence. qRT-PCR, Western blot, and immunocytochemistry analyses determined that adenoviral transduction efficiently inhibits the expression of MCT1. However, at 0.1 mM lactate, the concentration in which MCT1 largely contributes to transport rather than MCT4, transport studies revealed no reduction in incorporation or release of lactate. Using qRT-PCR and immunocytochemistry, increased MCT4 expression was observed in tanycyte cultures with MCT1 knock-down. This compensatory effect should be considered when using the AdshMCT1 for in vivo studies to assess changes in food intake.Grant support by FONDECYT 1100705, Center for Advanced Microscopy, CMA BIO BIO.

21.Adaptive mechanisms of salt tolerance in *Deschampsia antarctica* DESV. Gutiérrez, A.[1], **Tapia, D.**[1], Sandoval, A.[2], Bravo, L.[1], Gidekel, M.[1]. Programa de Doctorado en Ciencias m/ Biología Celular y Molecular Aplicada. Laboratorio de Fisiología y Biología Molecular Vegetal, Facultad de Ciencias Agropecuarias y Forestales. Universidad de La Frontera[1], Laboratorio de Fisiología y Biología Molecular Vegetal, Facultad de Ciencias Agropecuarias y Forestales. Universidad de La Frontera[2].
Soil salinity is a major factor limiting plant productivity, particularly those of agricultural significance. More than 800 million hectares of land throughout the world are salt affected, this accounts for more than 6% of the world's total land area. Salt Overly Sensitive pathway (SOS) plays an important role in salt tolerance of plants, controlling long- distances Na(+) transport over from root to the shoot. It consists of three proteins, SOS3, SOS2 and SOS1; SOS3 is a Calcium Sensors, activated under conditions of salt stress. Deschampsia antarctica, a Poaceae, surviving under adverse conditions in Antarctic territory. This plant has the ability to survive under hostile environmental conditions in this territory, as high salinity. This study contains SOS3 gene molecular analysis, anatomical and physiological evaluation of Deschampsia antarctica submitted to different treatments with NaCl (0.25 M a 1.0 M). The results show an increase SOS3 gene expression. Likewise also a high capacity for anatomical and physiological adaptability to the treatment conditions evaluated, demonstrating the formation of trichomes, increased levels of proline, stability of the photosynthetic apparatus, as well as an increase in the length of their leaf under saline treatments. Acknowledgements:Beca de apoyo de Tesis Doctoral CONICYT Código 24121490-2012DI12-008 Ex - convenio Desempeño II 2012. Dirección de investigación. Universidad de La Frontera.

22.Breakpoints region accessibility in RUNX1 gene is independent of histone acetylation. **Stuardo, M.**[1], Schnake, N.[1], Gutiérrez, S.[1]. Laboratorio de Regulacion transcripcional y Leucemia, Departamento de Bioquimica y Biologia Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción1.
One of most frequent chromosomal translocations found in about 15% of patients with acute myeloid leukemia is the t(8,21) that involves ETO and RUNX1 genes. RUNX1 breakpoints for this translocation are confined to intron 5 where it has been described the presence of three cleavage sites for topoisomerase II and one for DNase I. Previous results from our laboratory using promyeloid cells showed a differential pattern of histone H3 and H4 acetylation in RUNX1 intron 5. In fact, several regions were enriched in acetylated histones, when compared to an intron of the same gene in which no breakpoints has been described. Therefore, we hypothesize that histone acetylation may influence the accessibility of chromatin in intron 5 of the RUNX1 gene. To test this hypothesis we induced changes in the histone acetylation using histone deacetylase inhibitors (HDACi) in promyeloid cells (HL-60) and evaluated DNA accessibility to topoisomerase II and DNase I by quantitative real time PCR. Surprisingly, our results show that the accessibility to both enzymes is independent of histone acetylation, suggesting that other factor(s) regulates chromatin accessibility in this region.FONDECYT 1100670.

23.Polycystin-1 modulates mechanical stress-induced cardiomyocyte hypertrophy. **Fernández, C.**[1], Pedrozo, Z.[1,2], Hill, J.A.[3], Lavandero, S.[1,2,3]. Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/Faculty of Medicine.[1], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile.[2], Department of Internal Medicine, University of Texas Southwestern Medical Center, USA.[3].
Cardiac hypertrophy is an early biological process triggered by biomechanical stress and a critical factor in the development of cardiovascular diseases. Until now, identification of mechanosensors in cardiomyocytes remains uncertain. Polycystin-1 is a plasma membrane protein found in different cell types, including cardiomyocytes. Polycystin-1 has been described as a mechanosensor in kidney cells. We studied in the present work whether polycystin-1 could be a mechanosensor in cultured cardiac myocytes and regulates cardiac hypertrophy induced by mechanical stress. To this end, cultured cardiac myocytes were treated with hypo-osmotic solutions (mechanical stress inducer) or phenylephrine to trigger hypertrophy. A decrease in polycystin-1 was obtained by treatment with siRNAs specific for this protein. Both phenylephrine and hyposmotic stress increased [^{3}H]-leucine incorporation and β-myosin heavy chain (β-MHC) mRNA and protein in a time- and concentration-manner. In cardiac myocytes knock down for polycystin-1 with a specific siRNAs, phenylephrine increased all these parameters but hyposmotic stress was unable to do it. Together these results suggest that polycystin-1 induces cardiomyocytes hypertrophy when they are subjected to hyposmotic stress as a model of mechanical stretch and could be a new mechanosensor in cardiomyocytes. Supported by Anillo ACT1111 (SL), FONDECYT 3110039 (ZP) and CONICYT 24121238 (CF). CF holds a CONICYT fellowship.

24.Transcriptional factor FoxO1 and mitochondrial metabolism in cultured cardiomyocytes. **Quiroga, C.**[1], Riveros, C.A.[1], Lavandero, S.[1,2,3]. Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/Faculty of Medicine, Universidad de Chile[1], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile[2], Department of Internal Medicine, University of Texas Southwestern Medical Center, USA[3].
Mitochondrion is a key organelle in cell metabolism regulation. Our lab recently established that insulin induced mitochondrial fusion and increased oxygen consumption, mitochondrial membrane potential and ATP content. Insulin also negatively regulates the transcription factor FoxO1, another key regulator of glucose metabolism in hepatic, muscle, adipose tissue and heart. FoxO1 is over activated in pathological conditions and in the heart mediates the development of diabetic cardiomyopathy. We assessed the role of FoxO1 in the regulation of cardiomyocyte metabolism. The adenoviral overexpression of constitutive active FoxO1 (FoxO1CA) induced AKT phosphorylation. FoxO1CA decreased the mitochondrial membrane potential and oxygen consumption, but with an increase in ATP levels. The silencing of FoxO1 decreased ATP levels with a small increase in mitochondrial membrane potential. Taking together, these results suggest that FoxO1 could control metabolism in cultured cardiomyocytes, explaining the pathological consequences of its overactivation. Supported by Anillo ACT1111 and FONDECYT 1120212 (SL) and FONDECYT 3120220 (CQ).

25.Angiotensin (1-9): New pathways involved in the development of cardiac hypertrophy. **Sotomayor-Flores, C.**[1], Hechenleitner, J.[1], Rivera-Mejías, P.[1], López-Crisosto, C.[1], Parra, V.[1], Lavandero, S.[1,2,3]. Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences[1], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile[2], Department of Internal Medicine, University of Texas Southwestern Medical Center, USA[3].
Hypertension is a major cause of morbidity and mortality, both nationally and internationally for its clear relationship with the pathological stated known as cardiac hypertrophy. Within the main systems responsible for controlling blood pressure is the renin-angiotensin-aldosterone system (RAAS) that is responsible for the maintenance of the vascular tone and electrolyte balance. Recent findings indicate that the RAAS peptide, Angiotensin (1-9), has the ability to antagonize prohypertophic signals in cardiomyocytes. To understand the antihypertrophic mechanism of Angiotensin (1-9), we focus on investigating its effects on calcium signaling, since this ion plays a key role in the contraction and the control of cellular energy metabolism and gene expression in heart. The treatment of cardiomyocytes with 100 uM of Angiotensin (1-9) during 6 hours, did not have a direct effect on calcium movements, both cytoplasmic and mitochondrial, but decreased the calcium response of cardiomyocytes to norephineprine (a pro hypertrophic signal) and increased the mitochondrial calcium transients induced by histamine (which evokes the release of calcium from the endoplasmic reticulum to mitochondria). Finally, Angiotensin (1-9) also recovered the mitochondrial calcium response that is lost in cardiomyocytes pre-treated with norepinephrine, effect that is apparently caused by a decreased coupling between the reticulum and mitochondria. To our knowledge, this is the first study that relates the antihypertrophic effect of Angiotensin (1-9) with the control of the structural and functional coupling between both organelles. Anillo ACT1111 and FONDECYT 1120212 (SL). CLC holds a CONICYT fellowship.

26.Genetic and phenotypic characterization of an *Arabidopsis* sirtuin mutant. **Torres, E.**[1], Holzmann, C.[1], Montoya, P.[1], Jordana, X.[1]. Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.[1]. Sponsored by Jordana, X.
Sirtuins belong to a family of proteins highly conserved among diverse organisms from all kingdoms. Their enzymatic activity produces either deacetylation or ADP-rybosilation of target proteins, using NAD^+ as a cosubstrate. They have been proposed as regulators whose activity responds to changes in the NAD^+/NADH ratio, sensing the metabolic state of the cell and modulating processes such as transcriptional activity and key metabolic pathways. However, little is known about the two putative sirtuin genes present in plants and their function. We characterize an *Arabidopsis thaliana* insertional mutant of sirtuin 1 of which no homozygous plants can be obtained, and that presents alterations in gametophyte development. We analyzed the progeny of selfed *SRT1/srt1* plants where abnormal segregation ratios were observed, and where reciprocal crosses with wildtype plants show that both gametophytes can only partially transmit the mutated *srt1* alelle. When observing the seed condition on the siliques, we found a reduced seed set due to a high number of aborted ovules. Finally, using microscopy, we analyze the development of the male and female gametophytes, and the early embryo development, to assess in which stage sirtuin 1 acts as a key regulator. Financed by Fondecyt 1100601, Núcleo Milenio en Genómica Funcional de Plantas P10-062-F.

27.Bag3 modulates autophagy signaling pathways in Hela cells. **Rodríguez, A.E.**[1], Lavandero, S.[1,2,3]. Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/Faculty of Medicine, University of Chile[1], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile[2], Department of Internal Medicine, University of Texas Southwestern Medical Center, USA.[3].
In order to maintain protein quality, cells activate different degradative pathways to eliminate damaged and/or unfolded proteins. In one of those pathways, damaged proteins are trapped by double membrane vacuoles (autophagosomes) that subsequently are fused with lysosomes to degrade them. In this process known as autophagy, chaperones are required to select damaged proteins, but remains unknown whether chaperones may regulate autophagy activating signaling pathways. Here we studied whether the co-chaperone Bcl2-associated athanogene 3 (Bag3) regulates autophagy signaling pathways. To test this, Bag3 expression was silenced in HeLa cells using different shRNAs or siRNAs and autophagy induction was assessed by Western blot. In both silencing strategies, a decrease in the conversion of LC3I to LC3II was observed, suggesting that autophagy may be blocked. This reduction was also observed with the autophagy flux inhibitor bafilomycin. To determine which autophagy signaling pathways are blunted in HeLa cells knockdown for Bag3, Beclin1-Bcl2, mTOR and Atg5 protein levels were assessed by Western blot. Our data show that Bag3 silencing increases Beclin1/Bcl2 ratio while reduces phospho-mTOR/total-mTOR ratio, both effects are used to be associated to activation of autophagy. However, Bag3 silencing also decreases Atg5 protein levels, a key protein for LC3II lipidation. Our novel results suggest that molecular chaperone Bag3 regulates autophagy induction in HeLa cells. Supported by Anillo ACT1111 (SL) and CONICYT AT 24110051 (AR). AR holds a CONICYT fellowship.

28.Angiotensin-(1-9) counteracts mitochondrial fission induced by norepinephrine in cultured cardiomyocytes. **Rivera-Mejias, P.**[1], Pennanen, C.[1], Sotomayor-Flores, C.[1], López-Crisosto, C.[1], Parra, V.[1], Lavandero, S.[1,2,3]. Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/Faculty of Medicine[1], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile.[2], Department of Internal Medicine, University of Texas Southwestern Medical Center, USA.[3].
Cardiac hypertrophy (CH) is an adaptive response to several stimuli that raises contractile demand. Pathological CH is characterized by an irreversible increase in myocardial size and can be triggered by the catecholamine norepinephrine (NE). Mitochondria form a dynamic network whose morphology and function depend on fusion and fission process. We have recently shown that angiontensin-(1-9) is a novel antihypertrophic agent but it remains unknown whether this peptide may also regulate mitochondrial morphology. Therefore, cardiomyocytes were treated with NE (10 uM) for 24 h with or without angiotensin-(1-9) (100 uM, 0 - 24 h) and mitochondrial morphology was assessed using mitotracker green. Our results depict that NE induces mitochondrial fission in hypertrophied cardiomyocyte and decreases mitochondrial metabolism. Interestingly this process seems to be linked to CH because the induction of mitochondrial fission with a Mfn2 mutant increases cell size and contractile proteins. Mitochondrial fission generated by NE was prevented by treatment for 6 h with angiotensin-(1-9). Thus, NE and angiotensin-(1-9) produce opposite effects on mitochondrial dynamics, being the first report showing that angiotensin (1-9) controls mitochondrial morphology. Supported by Anillo ACT1111 and FONDECYT 1120212 (SL). CLC holds a CONICYT fellowship. .

29.Evaluation of RPRM on the induction of cell proliferation estrogen-mediated in cells lines of gastric cancer . **López, L.**[1,2], Marchant, M.[2,3], Corvalan, A.[2], Guzmán, L.[3]. Instituto de Química, Pontificia Universidad Católica de Valparaíso.[1], Centro de Investigaciones Médicas, Pontificia Universidad Católica de Chile.[2], Instituto de Química, Pontificia Universidad Católica de Valparaíso. [3].

Recent studies show that estrogen (E2) plays a key role in the pathogenesis of Gastric cancer (GC), where E2 enhances tumor proliferation through estrogen receptor in CG. Malik et al., (2010) show that E2 induces RPRM repression by recruiting of RPRM through ER alpha, in breast cancer. Based on the discussed above, our group assessed the effect of E2 on cell proliferation in a cell line AGS RPRM negative and AGS transfected stably with the coding region of RPRM (AGS RPRM +). ER alpha, ER beta expression and RPRM expression were evaluated in GC cell lines: AGS [RPRM(-) and RPRM(+)], and KATO-III by RT-PCR. We verified the methylation status of the promoter region through bisulfite sequence. in KATO III cells Finally, we have analyzed is the proliferative status by quantification of the ATP present in viable cells. We determined that all lines studied expressed RE alpha and beta and RPRM. In addition, was found that the rate of proliferation decrease a 54 % in AGS (RPRM+) in presence of 3 uM of E2 compared to the condition control AGS (RPRM+). These results suggest that endogenous expression of RPRM could be related with the inhibition of proliferation induced by E2 in gastric cancer cell lines. We should consider to measurement by qRT-PCR, the level expression of RPRM in AGS-RPRM+ cells treated with E2 and analyzed the RPRM protein by Western blot. Grant Support: 0337.460/2012 (PUCV), and Fondecyt-1111014.

30.Unexpected antagonistic effect of gossypol and resveratrol over the viability on human leukemic cell lines. **Cea, A.E.**[1], Ojeda, L.[1], Pérez, A.[1], Castillo, B.[1], Vega, E.[1], Zambrano, A.H.[1], Reyes, A.M.[1], Salas, M.R.[1]. Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile[1].

It is well known that natural products are a rich source of compounds for applications in medicine, pharmacy and biology. Gossypol is a natural phenol derived from the cotton plant (genus Gossypium), is a phenolic aldehyde that permeates cells and acts as an inhibitor for several dehydrogenase enzymes. It has proapoptotic properties, probably due to the regulation of the Bax and Bcl2. Resveratrol (RSV) a naturally occurring polyphenolic compound, is highly enriched in a variety of food sources, such as grapes, peanuts and red wine. RSV can inhibit the growth of human cancer cells in vitro by mechanism involved in apoptosis and cell cycle regulation. Since both reagents seams to act by different mechanism, we speculate that their combined use may yield a synergistic anticancer activity. By these reason we analyze the effect of Gossypol and RSV alone and in combination over the viability on human HL-60 and U937 leukemic cell lines. We used peripheral blood mononuclear cells (PBMC) as control. We found that Gossypol and RSV independly have a specific cytotoxic effect in both leukemic cell lines but they not affect control cells. The results of the combination of gossypol doses (up to 1,17 uM) and RSV (up to 24,3uM) show an antagonistic effect between both compounds in HL-60 and U937 cells. These unexpected result could be by the prooxidant effect of RSV at low doses. (Financed by FONDECYT 11090404, DID-UACh, FONDEF D07i1117).

31.Repression of Sall2 transciption factor by the p53 tumor supressor. **Farkas, C.A.**[1], Escobar, D.[1], Pincheira, R.[1]. Laboratorio de Transducción de Señales y Cáncer, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.[1].

The Sall2 transcription factor is a member of the SALL/SPALT gene family, involved in differentiation. Sall2 has been proposed as a tumor suppressor protein in ovarian cancer, yet Sall2 is highly expressed in other malignancies, including Wilm's tumor, oral and testicular cancers. The function of Sall2 including its target genes and regulatory mechanism are poorly characterized. We demonstrated that there is an inverse correlation between the expression of Sall2 and the p53 tumor suppressor. After genotoxic stress, p53 levels increase while Sall2 protein and mRNA decrease. This result has been found in various cell types. Here, we investigated regulatory mechanisms between Sall2 and p53. Bioinformatic analyses revealed putative p53 responsive elements along the Sall2 promoter, suggesting that Sall2 is a direct target of p53. We cloned Sall2 promoter sections in front of the luciferase reporter gene. Overexpression of wild type p53 or endogenous p53 activation with the chemotherapeutic agent doxorubicin repressed Sall2 promoter activity. Our data suggest that a functional p53 represses Sall2 transcription by affecting its promoter activity. However, direct binding of p53 to the Sall2 promoter must be tested. The regulation of Sall2 by p53 may be important in the cellular responses that abrogate cell proliferation during genotoxic insults. FONDECYT 1110821.

32.Role of Sall2 transcription factor in cell proliferation and survival after acute genotoxic injury. **Morales-Gedda, M.**[1], Sodir, N.[2], Nualart, F.[3], Pincheira, R.[1]. Laboratorio de Transducción de Señales y Cáncer, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.[1], Department of Pathology and Helen Diller Family Comprehensive Cancer Center, University of California San Francisco, USA.[2], Laboratorio de Neurobiología y Células Madres, Departamento de Biología Celular, Facultad de Ciencias Biológicas, Universidad de Concepción.[3].

Transcription factors are key cellular components in many biological process and their activities determine how cells function and respond to the environment. Thus, alteration in their normal function has been associated with diverse disorders, including cancer. Sall2 is a transcription factor that plays a role in neuronal development and neurogenesis. In addition, several evidences suggest it could also play a role in cancer However, little is known about Sall2 function and its involvement in human diseases. *In vitro* evidence on normal and cancer cells suggest that Sall2 plays a role in cell proliferation and survival. To determine a role for Sall2 in any of this process, we analyzed *in vivo* levels of proliferation and cell death in Sall2 deficient and wild type mice exposed to genotoxic insult. We collected tissues 6 hours after exposure to gamma radiation, and performed immunohistochemistry on matched samples of Sall2 wild type and null mice. Analysis of sections of small intestine showed that, in Sall2 deficient mice, the number of proliferative cells at the crypt is extremely low compared to the wild type tissues. Analysis of apoptosis in thymus and spleen sections showed that, in Sall2 deficient mice. the number of apoptotic cells significantly decreases compared to the wild type tissues. Thus, our studies demonstrated *in vivo*, significant differences between Sall2 deficient and wild type mice exposed to acute genotoxic stress, confirming a role for Sall2 in these cellular processes. FONDECYT 1110821.

33.Germ cell autophagy: adaptive response to serious glutathione deficiency. Mancilla, H.[1], Cereceda, K.[1], Maldonado, R.[1], Burgos, J.[1], López, C.[1], Montesdeoca, M.[1], Villarroel-Espíndola, F.[1], Castro, M.A.[1], Slebe, J.C.[1], Lavandero, S.[2], Concha, I.I.[1]. Instituto de Bioquímica y Microbiología, Universidad Austral de Chile[1], NEMESIS, Centro Estudios Moleculares de la Célula (CEMC), Universidad de Chile.[2]. Sponsored by Concha, I.I.

Autophagy is the major intracellular degradation system by which cytoplasmic materials are delivered and degraded in the lysosome. In fact, autophagy acts as a pro-survival mechanism during oxidative stress or nutrient deficiency. The development and survival of male germ cells depend on their close contact with Sertoli cells and the antioxidant capacity of the seminiferous epithelium. Glutathione is one of the most important antioxidant during spermatogenesis. In this work, we evaluated autophagy involvement in germ cell survival during severe glutathione deficiency. We showed that disruption of glutathione metabolism with buthionine sulfoximine (BSO) produces an important decrease of glutathione content in mouse germ cell lines GC-1 and GC-2, without producing cell death. Autophagy was assessed by processing and subcellular distribution of the endogenous protein LC3-I. Immunoblot and immunofluorescence analysis showed consistent increase of LC3-II and accumulation of autophagic vesicles in glutathione depleted conditions. This process involves the activation of AMP-activated protein kinase (AMPK), as well as increased glycogen degradation during the induction of autophagy in glutathione depleted cells. These results suggest that in glutathione deficiency, germ cells activate AMPK-dependent autophagy increasing testicular glycogen breakdown as an adaptive stress response.FONDECYT-1110508, 1090740, 1110571, FONDAP-1501006, Anillo de Investigación de Ciencia y Tecnología-10176. HM: Becario Doctorado CONICYT.

34.Endometrial expression of progesterone receptor in subgroups of obese women with differing potential risk for developing endometrial cancer. Barahona, C.[1], Albornoz, C.[2], Castro, F.[2], Gaete, F.[2], Peñaloza, P.[2], Celis, M.[2], Villavicencio, A.[1,3]. Instituto de Nutrición y Tecnología de los Alimentos (INTA), Universidad de Chile[1], Hospital Dr. Luis Tisné Brousse, Universidad de Chile[2], U-CANCER: Red de Medicina Traslacional en Cáncer, Universidad de Chile[3]. Sponsored by Quest, A.

Background: Enhanced endometrial proliferation correlates obesity to endometrial cancer (EC). Our laboratory identified cycling obese women *without EC* with differing endometrial proliferation levels and endometrial expression of estrogen receptor (ER): obese women with high proliferation and ER expression (O_{HP}) and obese women with low proliferation *and ER expression* (O_{LP}). Considering that endometrial proliferation and differentiation are regulated, respectively, by 17β-estradiol (E_2) and progesterone (P_4) through their receptors; we propose that these subgroups of obese women have different levels of endometrial expression of progesterone receptor (PR). *Goal:* Evaluate the endometrial expression of PR in those subgroups of obese patients. *Methodology:* Endometrial samples were obtained from cycling women in the proliferative phase (without EC) with body mass index (BMI) = 18-24.9 Kg/m^2 (normal-weight, N, n=8), BMI> 30 Kg/m^2 (obese, O_{HP}, n=6; O_{LP}, n=3) and obese women with type-I EC (O_{EC}, n=10). PR expression was measured by immunohistochemistry. *Results:* The expression of RP was lower in O_{EC} group than in O_{HP} and O_{LP} groups, respectively (49.3% and 45.6%, $p<0.05$). Interestingly, within O_{HP} group we found a subgroup of patients whose PR expression level was 30% lower ($p<0.05$) than the rest O_{HP} women and similar to O_{EC} patients. *Conclusions:* These data suggest the existence of a subgroup within O_{HP} women with PR levels similar to O_{EC}. Lower PR expression will not allow P_4 to counteract the proliferative effect of estrogens, suggesting that this subgroup will have higher risk of developing EC. FONDECYT-1110232.

35.Pannexins and Connexins are both required for Thy-1-induced astrocyte adhesion and migration. Alvarez, A.[1], Kong, M.[1], Quest, A.F.[1], Leyton, L.[1]. Laboratorio de Comunicaciones Celulares, Centro de Estudios Moleculares de la Célula. Facultad de Medicina. Universidad de Chile[1].

Our laboratory has shown that the neuronal protein Thy-1 induces an increase in the number and size of focal adhesions in astrocytes. In addition, sustained Thy-1 stimulation induces astrocyte migration. Both responses require an integrin-dependent increase in extracellular ATP that stimulates P2X7-receptor activation and Ca2+ entry. In astrocytes, ATP may be released through the hemichannels formed by Pannexins and Connexins, or by exocitosis. However, the mechanism by which ATP is released via Thy-1-engaged αvb3 integrin is unknown. Because Brefeldin-A did not affect Thy-1-induced ATP release, we evaluated whether Pannexins and/or Connexins were involved. Using DITNC-1 astrocytes as a model, extracellular ATP was quantified with a luminescence-based assay and hemichannels opening using a Lucifer yellow uptake assay. Calcium kinetics were evaluated with Fluo-4-AM. Indirect immunofluorescence analysis was employed to detect adhesions and cell migration was assessed in wound-healing assays.Treatments of DITNC-1 astrocytes with pharmacological inhibitors of Pannexins or Connexins reduced Thy-1-induced ATP release, intracellular calcium kinetics, focal adhesion formation and astrocyte migration. In addition, the inhibition of both hemichannels together, completely blocked the Thy-1-induced responses in astrocytes. Moreover, hemichannel opening required calcium release from the Endoplasmic Reticulum through the IP3 receptor (IP3R), since treatment with an IP3R inhibitor reduced ATP release and hemichannels opening. Thus, Thy-1-engaged αvb3 integrin induces ATP release upon intracellular calcium increase and requires both Pannexins and Connexins. Conicyt 21090323 and 24001198 (AA). FONDECYT 1110149 (LL), 1090071(AFGQ); FONDAP 15010006 (AFGQ); ICMP09-015-F (LL).

36.Recombinant expression of N-terminal end of isoform c from human Endothelin Converting Enzime-1 fused to Glutathione-S-Transferase. Niechi, I.[1], Maldonado, E.[2], Armisén, R.[2,3], Fernández, C.[3,4], Tapia, J.C.[1,2,3]. Cell Transformation Laboratory[1], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile[2], U-CANCER: Network for Translational Medicine in Cancer, University of Chile[3], Department of Anatomopathology, HCUCH[4].

Endothelin Converting Enzime-1 (ECE-1) is a membrane metalloprotease involved in endothelin-1 (ET-1) synthesis and its expression has been linked to many normal and pathological cell processes, including cancer. Isoform ECE-1c is the most abundantly expressed form in cancer cells and its over-expression leads to a malignant tumor phenotype. Noteworthy, ECE-1c has been related with the Wnt/β-catenin signaling pathway, which is directly involved in tumor development and progression. Interestingly, N-terminal end of ECE-1c displays four putative phosphorylation sites for protein kinase CK2, which is also linked to the Wnt/β-catenin pathway. These data suggest that ECE-1c, as a potential CK2 substrate, should be considered as a useful target for cancer diagnosis and treatment.The aim of this work was to express the recombinant N-terminal end of ECE-1c fused to GST, by which this sequence was amplified by PCR and cloned into the shuttle vector pGEM-T-easy. Then, it was subcloned fused to the 3' terminal end of Gst gene into the bacterial expression vector pGEX-2T. Recombinant GST-fused peptide expression was optimized in *E. coli* and then purified with a Glutathione-Agarose affinity column. Protein expression was analyzed by Coomassie blue staining of SDS-PAGE gels and confirmed by western blotting with a specific anti-GST-antibody.*Supported by the International Centre for Genetic Engineering and Biotechnology (ICGEB) CRP/CHI10-01, and Fondecyt 1120132 (to J.C.T). (For more information, please see poster from Eduardo Silva into this meeting)..*

37.Lithium chloride induces autophagy in seminiferous tubule cells via inositol monophosphatase. Cereceda, K.[1], Mancilla, H.[1], Burgos, J.[1], López, C.[1], Montesdeoca, M.[1], Villarroel-Espíndola, F.[1], Angulo, C.[1], Castro, M.A.[1], Concha, I.I.[1]. Instituto de Bioquímica y Microbiología, Universidad Austral de Chile[1].

Autophagy is a cellular process that maintains the homeostasis of the normal cell, allowing for cell survival in times of metabolic stress. Autophagosome formation is negatively regulated by the mammalian target of rapamycin (mTOR) pathway. Apart from mTOR-dependent pathway, mTOR-independent pathway has been described. In this process, the inhibition of inositol monophosphatase (IMPase) reduces free inositol and myoinositol-1,4,5-triphosphate levels, which leads to an upregulation of autophagy.It has been demonstrated that rat spermatocytes are able to undergo autophagy but there are no studies about autophagy regulated by IP3 in seminiferous tubule cells. In this study we treated Sertoli cells and germ cells with Lithium Chloride which is known to induce autophagy via Inositol Monophosphatase (IMPAse) in other cells types. We used Rapamycin as positive control and co-treated the cells with Bafilomicyn to prevent LC3 degradation. After these treatments, the induction of autophagy was assessed via LC3 II ratio by Western Blot, the increase in LC3 II levels suggests autophagy induction. The same treatments were evaluated by immunofluorescence to study the LC3 distribution, which was uniform in all cytoplasm in control cells but changed to a speckle pattern in treated ceils, suggesting the formation of autophagosomes. To confirm that LiCl induces autophagy via IMPAse cells were treated with L-690,330, an IMPase inhibitor and the results were similar to those observed in LiCl-treated cells. These results demonstrate the induction of autophagy regulated by mTOR-independent pathway in seminiferous tubule cells.FONDECYT 1110508, 1110571.

39.Characterization of Sall2-deficient mouse embryo fibroblasts. Riffo, E.[1], Pincheira, R.[1]. Laboratorio de Transducción de Señales y Cáncer, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.[1].

Sall2, a member of the Spalt gene family is a poorly characterized transcription factor involved in neurogenesis and differentiation. Interestingly, like other members of the family, Sall2 is deregulated in various cancers, which suggests it could play a role in this disease. However, the role of Sall2 in tumorigenesis and/or neoplastic progression is still controversial. We are using Mouse Embryo Fibroblasts (MEFs) isolated from wild-type and Sall2-deficient mice to understand Sall2's role in cellular functions. We immortalized wild-type and Sall2-deficient MEFs by infection with the wild-type simian virus 40 large T antigen, and analyzed their cellular behavior. Our data showed that lack of Sall2 increases proliferation rate of MEFs, incidence of foci formation, and anchorage-independent cell growth. In addition, Sall2-deficient cells showed increased cell death under low serum conditions. These data support a role for Sall2 as a tumor suppressor, and a survival factor under serum deprivation. We are currently investigating the molecular mechanisms involved in Sall2 *cellular function.* FONDECYT 1110821.

38.mTOR pathway regulation by the TrkA neurotrophin receptor; possible implications on neuronal differentiation. Fuentes-Villalobos, F.[1], Pincheira, R.[1], Castro, A.F.[1]. Laboratorio de Transducción de Señales y Cáncer, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.[1].

Normal development of an organism's system is a complex and organized process, controlled by external and internal signals contributing to cell fate decisions. Nervous system requires building itself from precursor cells that proliferate, migrate and differentiate into functional units, like neurons. In mammals, this process is acutely mediated by neurotrophins, a family of proteins that is also needed in adult life. Nerve Growth factor (NGF), a prototypic neurotrophin, interacts with TrkA and $P75^{NTR}$ receptors. TrkA activation by NGF induces neuronal differentiation through a transduction cascade in which participate Ras, PI3K and PLC-γ1. In primary afferent neurons, NGF, through an unknown mechanism, activates the mTORC1 pathway that mainly promotes protein translation. We found that specific inhibition of TrkA function with K252a, reduced NGF-dependent mTORC1 activation in PC12 cells ($TrkA^{+/+}$) a model for studying TrkA-dependent neuronal differentiation. Consistent with a role for mTORC1 activation in neuronal differentiation downstream of TrkA, rapamycin reduced NGF-induced differentiation of PC12 cells. To confirm that NGF activates mTORC1 through TrkA, we used PC12nnr5 cells ($TrkA^{-/-}$). Interestingly, the downstream target of mTORC1, S6K, is constitutively activated in PC12nnr5 cells, while activation levels of the TrkA downstream targets AKT and ERK are extremely low. Thus, our results suggest that TrkA modulates mTORC1 pathway to induce normal differentiation of neurons. To better understand this mechanism, we are investigating whether known modulators of the Akt-mTOR pathway, such as DISC1 and Girdin, are involved in the TrkA-dependent regulation of mTORC1. FONDECYT 1120923.

40.Ceramides-induced insulin signaling desensitization is linked to mitochondrial network fragmentation. López-Crisosto, C.[1], Parra, V.[1], Castro, P.[2], Lavandero, S.[1,3,4]. Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/ Faculty of Medicine, University of Chile[1], Faculty of Medicine, P. Catholic University of Chile[2], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile[3], Department of Internal Medicine, University of Texas Southwestern Medical Center, USA[4].

Overweight and obesity are highly prevalent conditions world-wide that represent important risk factors for the development of diabetes and cardiovascular diseases. Lipid oversupply and lipotoxicity are key factors involved in these complications. It is well known that ceramides, derived from lipid metabolism, contribute to insulin resistance. Cardiac diseases are the main death cause in diabetic patients; therefore a better understanding of the mechanisms leading to cardiac damage and insulin resistance is required. Cardiomyocytes consume large amounts of ATP, produced through oxidative phosphorylation in the mitochondria, as an energy source for their contractility. Mitochondria form a dynamic network that is constantly remodeled by fission and fusion events. We investigate here the effects of ceramides on insulin signaling and mitochondrial dynamics in cultured rat cardiomyocytes. Treatment of cardiomyocytes with C_2-ceramide (40 μM, 3 h) decreased phospho-Akt levels in response to insulin. C_2-ceramide also induced mitochondrial network fragmentation, increasing the translocation of the mitochondrial fission protein Drp-1 to mitochondria. To test whether both effects were linked, the chemical inhibitor of Drp-1, mdivi-1, was added before treatment with C_2-ceramide. Mdivi-1 prevented mitochondrial fragmentation caused by C_2-ceramide and rescued insulin signaling in cardiomyocytes. In conclusion, the decrease in Akt phosphorylation in response to insulin caused by ceramides appears to require mitochondrial fission. Supported by Anillo ACT1111 and FONDECYT 1120212 (SL) and FONDECYT 1090727 (PC). CLC holds a CONICYT fellowship.

41.Herp regulates autophagy through Beclin-1 lys^{48} poly-ubiquitination and proteasomal degradation. Gatica, D.[1], Paredes, F.[1], Quiroga, C.[1], Troncoso, R.[1], Pedrozo, Z.[1,2], Lavandero, S.[1,2,3]. Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/Faculty of Medicine.[1], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile. [2], Department of Internal Medicine, University of Texas Southwestern Medical Center, USA[3].

Autophagy is an important self-degradation process in the cell. Beclin-1/ATG6 is a key protein for autophagy initiation and development. Recent reports show that Beclin-1 is degraded by the ubiquitin-proteasome system. Herp is an endoplasmic reticulum (ER) transmembrane protein containing an ubiquitin-like domain linked with ER-associated protein degradation. We have shown that Herp is a novel negative regulator of autophagy. Because Herp regulates the poly-ubiquitination of certain proteins substrates, we investigate its role on Beclin-1 proteosomal degradation via Lys^{48}-linked poly-ubiquitination. Beclin-1 Lys^{48}-linked poly-ubiquititination was determined by overexpressing Flag-Beclin-1 in Herp knock-down (KD) and control HeLa cells followed by immunoprecipitation of Flag and Western blot against Lys^{48}-linked ubiquitin. Proteasome activity was inhibited with MG132 for 6 h and Beclin-1 levels were assessed by Western blot in Herp KD cells or HeLa WT cells treated with Herp siRNA. Autophagy was evaluated by LC3-II Western blot in Herp siRNA treated HeLa WT cells. GFP-LC3 dots were analyzed in Herp KD U2OS GFP-LC3 cells.Our results show that Lys^{48}-linked poly-ubiquitination of Beclin-1 is decreased in Herp absence. Proteasomal inhibition increased Beclin-1 levels in control HeLa cells but not Herp KD cells. Autophagy was upregulated in Herp siRNA treated cells. Altogether, our results suggest that Herp controls autophagy by reducing Beclin-1 levels in an ubiquitin-proteasome dependent manner.Supported by Anillo CT1111 and FONDECYT 1120212 (SL), FONDECYT 3110114 (RT), FONDECYT 3120220 (CQ) and FONDECYT 3110039 (ZP). FP holds a CONICYT fellowship.

42.Modulation of glycogen synthesis in male germ cells treated with lithium. Salazar, E.[1], Villarroel-Espíndola, F.[1], Angulo, C.[1], Castro, M.A.[1], Slebe, J.C.[1], Ramirez, A.[2], Concha, I.I.[1]. Instituto de Bioquímica y Microbiología, Universidad Austral de Chile[1], Instituto de Ciencia Animal, Universidad Austral de Chile[2]. Sponsored by Concha, I.I. Lithium salts are commonly used for treating severe psychiatric disorders, however, it causes various side effects, including male reproductive toxicity. Lithium is a known glycogen synthase kinase 3beta (GSK3beta) inhibitor. This enzyme regulates by phosphorylation the enzyme responsible for glycogen synthesis, glycogen synthase (GS). In this work we determined the phosphorylation states and subcellular localization of GS and GSK3beta in germ cells lines GC-1 and GC-2 and equine spermatozoa treated with 30 mM lithium chloride. Using Western blot analysis and immunofluorescence we observed that lithium lowers the level of phosphorylation at serine 640 of GS in both cell lines, in nucleus and cytosol. GSK3beta, however, presented an increased phosphorylation at serine 9 (GSK3beta inactive), in contrast with the decreased phosphorylation at tyrosine 216 (GSK3beta active). In spermatozoa we determined an apparent increase in phosphorylated GSK3beta at serine 9, without changes in the total level of this enzyme. and an increase in the immunodetection of GS as a result of the capacitation, which was reversed by lithium. We also observed an increase in the amount of glycogen in cells GC-1, GC-2 and uncapacitated spermatozoa. These results suggest that multiple side effects described for lithium on seminiferous epithelium cells could be explained by changes observed in glycogen metabolism.FONDECYT-1110508, 1090740, 1110571.

43.Insulin reverses gestational diabetes-increased L-arginine transport involving A_{2A} adenosine receptors activation in human umbilical vein endothelium. Guzmán-Gutierrez, E.[1], Westermeier, F.[1], Salomon, C.[1], Arroyo, P.[1], Pardo, F.[1], Leiva, A.[1], Sobrevia, L.[1]. Cellular and Molecular Physiology Laboratory (CMPL), Division of Obstetrics and Gynecology, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile.[1].

Gestational diabetes (GD) associates with higher expression and activity of cationic amino acid transporters 1 (hCAT1) in human umbilical vein endothelial cells (HUVEC), phenomenon involving A_{2A} adenosine receptor (A_{2A} AR) activation and increased hCAT-1-mediated L-arginine transport. We evaluated whether insulin reverses GD-increased L-arginine transport and hCAT-1 protein abundance involving A_{2A} AR. L-Arginine transport (31-1000 uM, 3 uCi/ml, 37°C, 1 minute) and hCAT-1 protein abundance was measured in HUVEC from normal (Nc) or GD (GDc) pregnancies. Cells were preincubated (8 hours) with insulin (1 nM) +/- ZM-241385 (10 nM, A_{2A} AR antagonist) or nitrobenzylthioinosine (NBTI, 10 uM, adenosine uptake blocker). GD associates with higher maximal velocity (without apparent Km alteration) for L-arginine transport and hCAT-1 protein abundance. Insulin blocked these alterations in GDc, effect abolished by NBTI. Insulin increased transport and hCAT-1 protein abundance in absence or presence of NBTI in Nc. NBTI did not alter transport and hCAT-1 protein abundance in GDc, but increased these parameters in Nc without insulin. NBTI increased transport and hCAT-1 protein abundance in Nc, an effect blocked by ZM-241385. However, hCAT-1 protein abundance did not change in GDc In presence of NBTI+ZM-241385. ZM-241385 also blocked GD and insulin effect in Nc on transport and hCAT-1 protein abundance. In conclusion insulin could be a factor reversing GD to normal fetal endothelium phenotype requiring A_{2A} AR activation. CONICYT (ACT-73 PIA, AT-24120944), FONDECYT (1110977, 11110059). CONICYT-PhD fellowship (EG-G, PA, CS), PUC-Faculty of Medicine PhD fellowship (CS, PA).

44.Synergistic cytotoxic effect of resveratrol and nordihydroguaiaretic acid over human HL-60 and U937 leukemic cell lines. Parada V. , D.[1], Ojeda O., L.[1], Ferreira P., J.[2], Jaña P., F.[3], Reyes P., A.[1], Salas G., M.[1]. instituto de Bioquimica y Microbiología, Facultad de Cs., Universidad Austral de Chile[1], Programa de Farmacología Molecular y Clínica, facultad de Medicina, Universidad de Chile[2], Programa de Farmacología Molecular y Clínica, Facultad de Medicina, Universidad de Chile[3].

The formulation of drug combinations could provide increased effectiveness in therapeutics, decreasing dose and avoiding drug toxicity. We assessed combinations of resveratrol (RSV) and nordihydroguaiaretic acid (NDGA) over human leukemic cells, as new prospective therapeutic protocols for this illness. RSV and NDGA are natural polyphenolic compounds with antioxidant and pro-oxidants properties. We determined the individual and combined effect of these compounds on cell viability, ATP levels, the activity of caspase-3 and detection of apoptosis by flow cytometry using double staining with annexin V-FITC/PI on HL60 and U937 leukemic cell lines. RSV and NDGA exerted a specific cytotoxic effect on both cell lines, not affecting normal cells (PBMC), and their combination produced a synergistic effect, more marked in the line U937. These compounds also inhibited ATP levels, increased caspase-3 activity and induced apoptosis on both leukemic cell lines. The combination induced a synergistic effect on HL-60 cells but an additive one on U937 cells. Cytotoxicity on these cells does not seem to be caused by decreasing levels of ATP, instead appears to be dependent on caspases activation. (Financed by FONDECYT 11090404 and 1090075, DID-UACh and FONDEF D07i1117).

45.The use of antisense oligonucleotides complementary to antisense non-coding mitochondrial RNA, as a novel therapeutical possibility for control of bladder cancer. Rivas, A.[1,2], Bendek, M.[1,2], Lobos-González, L.[2], Ávila , M.[1,2], Villegas, J.[1,2,3], Burzio, L.O.[1,2,3], Landerer, E.[2,4]. Facultad de ciencias biológicas, Universidad Andrés Bello[1], Andes Biotechnologies[2], Fundación Ciencia y Vida[3], Facultad de medicina, Universidad Andrés Bello[4].

Urothelial carcinoma of bladder is the seventh common cancer worldwide. In 2008, the prevalence of bladder cancer in USA was 537.000 patients. The treatment for the superficial presentation of this neoplasm it is transurethral resection (TURB). The 80% of patients subjected to TURB will develop a tumor recurrence with progress to muscular invasion. In the early 2000, our laboratory described the existence of a novel family of non-coding mitochondrial RNAs (ncmtRNAs), named Sense (S-ncmtRNA) and Antisense (AS-ncmtRNA). These molecules exhibit a differential pattern of expression according to malignancy and proliferative status of analyzed cells. Normal quiescent cell do not express this ncmtRNAs, normal proliferating cells express both ncmtRNAs and tumour cells selectively repress the expression of AS-ncmtRNA. The murine bladder cancer cells MB49 derived from a C57BL/6 mouse administered 7, 12-Dimethylbenz(a)anthracene, were treated with antisense oligodeoxynucleotides (AS-ODNs) complementary to these mitochondrial transcripts *in vitro*. This treatment caused massive cell death, decreased proliferative capacity and diminished wound healing closure compared with those treated with a control AS-ODN and non-treated cells. Furthermore, these treatments with AS-ODNs in normal cells, do not induce cell death. Taken together these results support a new therapeutic strategy for bladder cancer. Supported by FONDECYT 11100385 (EL) and Andes Biotechnologies S.A.

46.Development of a premium variety of apple seedlings with greater sweetness. Díaz, F.[1], Aguayo, F.[1], Mandujano, P.[1], Zamudio, S.[1], Araya, J.[1], Arcos, Y.[2], Arce, P.[2], Norambuena, L.[1], Stange, C.[1], Handford, M.[1]. Laboratorio de Biología Molecular Vegetal, Facultad de Ciencias, Universidad de Chile[1], Laboratorio de Bioquímica, Departamento de Genética Molecular y Microbiología, P. Universidad Católica[2].

Sorbitol is a sugar alcohol and is the major phloem-translocated carbohydrate in Rosaceae species, such as apples, pears, nectarines and cherries. In these species, sorbitol dehydrogenase (SDH) catalyses the reversible oxidation of sorbitol, which has low sweetening power, to fructose, a sugar with enhanced sweetness. This makes SDH an excellent candidate for modulating naturally the composition of sugars in the fruit, if specifically over-expressed in this organ. With the aim of generating a new variety of apples with sweeter fruits, different binary vectors were generated in which SDH has been cloned under the control of two different promoters, one constitutive and the other fruit-specific. To achieve our aim, we have used tomato plants as a model system to test the functionality of our vectors. Firstly, using biochemical analyses, we show that there is greater SDH activity in extracts of fruits which have been transiently transformed with our vectors compared to control-transformed fruits. Secondly, tomato plants were stably transformed with *Agrobacterium tumefaciens* carrying the vectors, and molecular analyses such as PCR and RT-PCR, have been performed to determine the expression of the transgenes in leaves. Finally, we are successfully implementing a system for the stable transformation of apples, and have transformed explants with *A. tumefaciens* harbouring a control vector, pBI121 carrying the *uidA* reporter gene. Funding: Innova-Corfo 07CN13PBD-19.

47.Cyclooxygenase-2 up-regulates the expression of Endothelin Converting Enzyme-1 via prostaglandin E2 production in colon cancer cells. Silva, E.[1], Cataldo, R.[1], Armisén, R.[2,3], Fernández, C.[3,4], Tapia , J.C.[1,2,3]. Cell Transformation Laboratory[1], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile[2], U-CANCER: Network for Translational Medicine in Cancer, University of Chile[3], Department of Anatomopathology, HCUCH[4].

Over-expression of cyclooxygenase-2 (COX-2) in colon cancer cells leads to an increased production of prostaglandin E2 (PGE2), which subsequently enhances the β-catenin activity and hence up-regulates genes promoting metastasis. Endothelin Converting Enzyme-1 (ECE-1) is crucial in the endothelin-1 production and displays known cancer-related effects in several cell models. Thus, we studied whether the COX-2/PGE2/β-catenin axis can also up-regulate the ECE-1 expression.Up-regulation of β-catenin activity in human normal (HEK-293T) and colorectal cancer (DLD-1) cells was performed by ectopically expressing β-catenin, CK2α, AKT-CA (constitutively active form), COX-2, and alternatively incubating with PGE2 or a GSK3β inhibitor (SB-216763). Down-regulation was performed by expressing AKT-DN (dominant negative form), siRNA specific for CK2α subunit, and incubating with specific inhibitors for CK2 (TBB) or COX-2 (SC-791). Then, ECE-1, COX-2, survivin and β-catenin mRNA and protein levels were analyzed by RT-PCR and western-blot, respectively.ECE-1 mRNA and protein levels did change after regulation of β-catenin activity with PGE2 in a similar way what happened with COX-2 and survivin in HEK-293T and DLD-1 cells.Our results indicate that the COX-2/PGE2/β-catenin axis indeed up-regulates the expression of ECE-1, strongly suggesting that the latter may have a strong impact on the malignant potential of colon cancer cells.*Supported by the International Centre for Genetic Engineering and Biotechnology (ICGEB) CRP/CHI10-01, and Fondecyt 1120132 (to J.C.T). (For more information, please see poster from Ignacio Niechi into this meeting).*

48.Identification and characterization of pollen-specific promoters in *Arabidopsis thaliana*. Muñoz, D.[1], León, G.[1]. Laboratorio de Reproducción y Desarrollo de Plantas, Centro de Biotecnología Vegetal, Universidad Andrés Bello[1].

The transition from a vegetative to a reproductive program in plants is accompanied by a massive transcriptional remodeling, evidencing the beginning of the gametophyte genetic program. It has been determined that about 14,000 genes are expressed during the development of the male gamete (pollen), and 5% of these genes are thought to be pollen specific. With the aim to identify pollen-specific promoters, we have identified genes that are expressed exclusively in pollen. To this, we use microarrays databases to identify genes that accomplish two criteria: i) they are not expressed in vegetative tissues and ii) their transcripts are detected only in developing pollen after the first mitosis. Using these searching criteria we identify the best 5 candidate genes and confirm the microarray data using RT-PCR. On the other hand, the putative promoter regions (500 base pairs upstream the start codon) of these genes were analyzed *in silico*, searching for overrepresented sequences, some of which are described in the promoter region of a pollen specific gene (LeLat52). Analysis of transgenic plants expressing a reporter gene (*GFP/GUS*) under the transcriptional control of these putative promoters allowed us to determinate the spatial and temporal expression pattern, in order to identify promoter sequences highly specific for pollen. Funded by FONDECYT Grant 1120766 and UNAB DI 74-12/R.

49.Development of a Strand Specific RT-PCR Protocol. Cerda, C.[1], Munita, R.[1], Gysling, K.[1]. Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile[1]. Sponsored by Canessa, P.

The RT-PCR technique is a variant of the traditional PCR, which allows the reverse transcription from RNA to complementary DNA (cDNA). The RT-PCR has great advantages such as high sensitivity, detection specificity and the possibility of being quantitative. However an important weakness is that this technique is not strand specific. In order to solve this weakness, it has been used some protocols that try to identify single strand transcripts specifically, but all of them are not effective enough and present different experimental artifacts that generate a lack of specificity in the detection. In this work, we report the development of an efficient protocol of tagged RT-PCR that is selective for the identification of a single-stranded RNA. To improve the existing protocols we elaborated solutions to each of the artifacts present in the classic reverse transcription technique. For this purpose, we used Actinomycin D in the reverse transcription step to inhibit DNA-dependent DNA polymerase (DdDP) activity of the reverse transcriptase. We also added an enzymatic blocking of the 3' end of the cDNA and the primers. We consider that this new protocol can be very useful in the identification and characterization of many kinds of natural antisense transcripts (NATs), and in the detection of single-stranded RNA viral genomes. Funded by the grants ICMP10-063-F and FONDECYT 1110392.

50.Functional characterization of pollen-specific kinases through the generation of knock-down transgenic plants expressing hairpin-RNAs (hpRNAs). Lucca, N.[1], García, M.P.[1], León, G.[1]. Laboratorio de Reproducción y Desarrollo de Plantas, Centro de Biotecnología Vegetal, Universidad Andrés Bello[1].

Pollen grains are the male gametophyte of plants and thus are essential for plant reproduction and productivity. However, despite their biological and agronomical importance, little is known about the molecular mechanisms that regulate its development and function. Pollen development is a highly complex process, involving several cellular activities and ending with the production of mature pollen grains. In Arabidopsis, three cells compose mature pollen grains: a large vegetative cell and two small sperm cells engulfed in the cytoplasm of the vegetative cell. During fertilization, the vegetative cell must germinate and produce a pollen tube, a growing tip structure that directionally transports the sperm cells to the ovule to produce the double fertilization event.Currently, little is known about signal transduction pathways and molecular components involved in these processes. Using microarray data we have previously identified 4 genes encoding kinases proteins (*PSK1* to *4*, for *POLLEN SPECIFIC KINASE*) that are expressed exclusively during the last stages of pollen development, germination and tube elongation. To analyze the physiological relevance of these genes, we have generated transgenic plants expressing specific ihpRNAs for each one of these genes under the control of a strong promoter (35S) and a pollen-specific promoter (LAT52). Also, we have determined the sub-cellular localization of each kinase protein using 35S:*PSK*:*GFP* constructions in agroinfiltration experiments. Funded by Fondecyt 1120766 and UNAB DI-74-12/R.

51.Regulation of testicular glycogen metabolism: malin-laforin complex interacts with muscle glycogen synthase and PTG. Vander Stelt, K.[1], Villarroel-Espíndola , F.[1], Maldonado , R.[1], López, C.[1], Angulo, C.[1], Castro, M.A.[1], Slebe, J.C.[1], Concha, I.I.[1]. Instituto de Bioquímica y Microbiología, Universidad Austral de Chile[1]. Sponsored by Concha, I.I..

Glycogen is a branched polymer of glucose associated with a population of proteins that mediate its biological functions, Glycogen synthesis is a highly regulated process in which Glycogen Synthase (GS) exhibits changes in its subcellular localization, activation by glucose-6-phosphate, inactivation via phosphorylation and degradation by malin-laforin complex, newly discovered regulatory mechanism, which ubiquitinates GS and proteins involved in glycogen synthesis. To determine if malin and laforin play a regulatory role in glycogen synthesis in seminiferous epithelium we used spermatogonium cell line GC-1, Sertoli 42GPA9 and rat testis extracts. By RT-PCR, immunocytochemistry and Western blot analysis we determined the expression of the muscular isoform of GS (MGS), laforin and malin in these cells. Confocal microscopy showed that malin and laforin colocalize with MGS and also with phosphatase PP1. GST-pull down assays using the malin-ring domain as bait demonstrated the interaction between malin and endogenous MGS in cell line GC-1, Sertoli cell line 42GPA9 and whole testis confirming the formation of a complex that interacts with MGS and also with protein targeting to glycogen (PTG).These results suggest for the first time that in testis, glycogen synthesis is being regulated by a malin-laforin complex.(FONDECYT-1110508, 1090740, 1110571).

52.Incretin GLP-1 promotes functional endoplasmic reticulum-mitochondria coupling in vascular smooth muscle cells. Morales, P.E.[1], Torres, G.[1], Michea, L.[2], Lavandero, S.[1,2,3], Chiong, M.[1]. Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/Faculty of Medicine[1], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile[2], Department of Internal Medicine, University of Texas Southwestern Medical Center, USA[3].

In recent years, incretin hormone analogs have been proven as a successful treatment for diabetes. In diabetic patients, vascular smooth muscle cells (VSMC) switch to a proliferative and secretor phenotype, promoting progression of cardiovascular complications. These changes have been recently correlated with increased distance between endoplasmic reticulum (ER) and mitochondria. Given that the incretin GLP-1 inhibits vascular remodeling and promotes vasodilatation, we tested the effects of GLP-1 on ER-mitochondria coupling on the A7r5 rat aortic cell line. Here we show that GLP-1 100 nM increases Mitofusin-2 levels, a protein required for ER-mitochondria tethering. Moreover, we prove that GLP-1 promotes colocalization of these organelles by inmunofluorescence of ER- and mitochondria-stained cells, an effect not restricted to a particular cellular area, as evaluated by Manders coefficients. We measured mitochondrial Ca^{2+} levels by confocal microscopy of Rhod-FF treated cells, and observed that GLP-1 facilitates reticular Ca^{2+} entry to mitochondria, evaluated as a faster increase and higher levels of Ca^{2+} in mitochondria after histamine stimulation. These data show that GLP-1 promotes functional coupling of ER and mitochondria, suggesting a novel mechanism for incretin action on VSMC. Supported by FONDECYT 1110180 (MC), Anillo ACT1111 (SL, MC). PM and GT hold a CONICYT fellowship.

53.Glucagon like peptide-1 (GLP-1) modulates mitochondrial dynamics and metabolism in vascular smooth muscle cells A7r5. **Torres, G.**[1], Morales, P.E.[1], Michea, L.[2], Lavandero, S.[1,2,3], Chiong, M.[1]. Center for Molecular Studies of the Cell, Faculty of Chemical and Pharmaceutical Sciences/Faculty of Medicine[1], Institute of Biomedical Sciences, Faculty of Medicine, University of Chile[2], Department of Internal Medicine, University of Texas Southwestern Medical Center, USA[3].

The incretin GLP-1, used in diabetes treatment, promotes glucose metabolism in several cell types. Glucose metabolism can be regulated by mitochondrial dynamics. Modification of mitochondrial metabolic parameters by mitochondrial fission and fusion have been described in different cell types, but not in vascular smooth muscle cells (VSMC). We investigate here whether GLP-1 controls mitochondrial dynamics and metabolism on the rat aortic VSMC cell line A7r5. Cells were exposed to GLP-1 for different times and then loaded with mitotracker orange. Multi-slice imaging reconstitutions were obtained by confocal microscopy to assess mitochondrial morphology, and fusion was evaluated by quantifying the number and volume of particles per cell. Mitochondrial potential and ROS were measured by flow cytometry using JC-1 and dihydrochlorofluorescein, respectively. ATP was determined by luciferin/luciferase assay. GLP-1 (100 nM, 0.5 h) increased 1.4 +/- 0.2 fold the mitochondrial potential with no significant increase in total intracellular ATP content. Increases in ROS (1.5 +/- 0.1 fold over control) were observed only after 6 h GLP-1 exposure. In summary, these results suggest that GLP 1 promotes morphological changes in mitochondrial network and metabolism. Supported by FONDECYT 1110180 (MC); ANILLO ACT1111 (MC and SL) and CONICYT thesis support fellowship 24110120 (GT). GT and PM hold a CONICYT fellowship.

54.Antioxidant and antimicrobial capacity in leaves and fruit of Peumo (*Cryptocarya alba*) and Arrayan (*Luma apiculata*) tree species. **Fuentes, L.**[1], Franco, W.[2], Valdenegro, M.[1], Gómez, M.G.[3], Millon, C.[3], Martínez, J.P.[4], Figueroa, C.[5]. Centro Regional de Estudios en Alimentos Saludables (CREAS), Valparaíso, Chile[1], Departmento de Ingeniería Química y Bioprocesos, Pontificia Universidad Católica de Chile, Santiago, Chile[2], Departmento de Ingeniería Química y Ambiental, Universidad Técnica Federico Santa María, Valparaíso, Chile[3], Laboratorio de Fisiología y Biología Molecular Vegetal, INIA-La Cruz, La Cruz, Chile[4], Facultad de Ciencias Forestales, Universidad de Concepción, Chile[5].

A common sustainable management strategy is to use native plants in the food or nutritional industry. However, little is known about the biomolecules content in leaves and/or fruit of those plants. Samples from native species of Chile, Peumo (*Cryptocarya alba*) and Arrayan (*Luma apiculata*) trees, were collected from Valparaiso and Biobio Region, respectively. In order to elucidate the health potential of these species, the antioxidant capacity (AC) and total polyphenol content (TPC) was determined in leaves and fruit and these values were compared with the blueberry ones. In addition, the leaf and fruit extracts were tested against gram positive and gram negative bacteria in order to determine their potential antimicrobial activity. Antioxidant activity for both native species, determined by TEAC and DPPH activity, showed greater TPC in both native fruits. Arrayan fruit showed the highest AC, compare it with blueberry fruit. In general, fruit extracts had greater antimicrobial activity than leaves extracts. Arrayan fruits extracts were particularly efficient in inhibiting both gram positive and gram negative *Staphylococcus aureus* at minimum concentrations of 143 mg. Preliminary results are promising and may contribute to the introduction of new sources of foods rich in antioxidants.Work funded by Research Fund for Native Forest CONAF 064/2011 and, "Regional Program CONICYT-Creation of Regional Centers (Project No.R06i1004).

55.Evaluation of polyamidoamine (PAMAM) dendrimers as drug carriers using Tramadol and Morphine as model drugs. **Zúñiga, M.**[1], Vergara-Jaque, A.[1], Carrasco, V.[2], Comer, J.[3], Sandoval, C.[3]. Centro de Bioinformática y Simulación Molecular. Universidad de Talca[1], Laboratorio de Sintesis Asimétrica. Universidad de Talca[2], Centro de Bioinformática y Biología Integrativa. Universidad Andrés Bello.[3]. Sponsored by González,W.

Nanotechnology has provided new approaches to biomedicine and pharmacology, specifically related to the development of nanocarriers used to transport drugs. PAMAM dendrimers are widely studied as an optimal means for controlled drug delivery; however, their highly charged cationic surfaces have cytotoxic effects and therefore limit their clinical application. The functionalization of the surface groups by acetylation or by the addition of folic acid is proposed as a convenient strategy to neutralize the peripheral amine groups and improve dendrimer biocompatibility. Moreover, such groups may improve the encapsulation of the potent analgesic drugs Tramadol and Morphine. Due to their limited bioavailability and side effects, controlled release of these drugs, by a nanocarrier such as functionalized PAMAM, would be desirable. In this work, we determine the structural and energetic properties that facilitate the encapsulation of Tramadol and Morphine by unmodified and functionalized PAMAM-G5 dendrimers. For this purpose, we employed molecular dynamics simulations to analyze the binding of both drugs to these dendrimers and to characterize the mechanism of encapsulation at atomic level. The relative binding affinity of the PAMAM-drug complexes was determined by the MM-GBSA method. Our results correlate well with experimental data, confirming that Tramadol and Morphine are better encapsulated by functionalized PAMAM dendrimers than by unmodified PAMAM, suggesting that the modifications may both improve encapsulation and decrease toxicity. Moreover, the simulations further reveal that hydrogen-bond and electrostatic interactions govern the affinity of both drugs, showing better affinity for Morphine than Tramadol.

56.Acetaldehyde as a mediator of ethanol reinforcement: Determination of acetaldehyde levels formed *in vivo* in the ventral tegmental area of rat brain following acute ethanol administration. **Buscaglia, M.**[1,2], Quintanilla, M.E.[1], Rivera-Meza, M.[1], Herrera-Marschitz, M.[1,3], Morales, P.[1], Israel, Y.[1,2]. Programme of Molecular and Clinical Pharmacology, Institute of Biomedical Sciences (ICBM), Faculty of Medicine, University of Chile.[1], Department of Pharmacological and Toxicological Chemistry, Faculty of Chemical and Pharmaceutical Sciences, University of Chile.[2], Millenium Institute (BNI)-Chile.[3]. Sponsored by Sapag, A.

Several studies suggest that ethanol acts as a pro-drug, and that its first metabolite, acetaldehyde, mediates its reinforcement effect by stimulating dopaminergic neurons of the ventral tegmental area (VTA). It is not known yet whether acetaldehyde is generated in the VTA by acute ethanol administration, reaching concentrations sufficient to produce reinforcement. The purpose of the present study is to detect acetaldehyde production in rat brain, and to determine the levels achieved in the VTA following acute ethanol administration. Acetaldehyde was measured by Western blot, using its property to interact with proteins, forming protein-acetaldehyde adducts in the presence of a reducing agent. Adducts were then detected with polyclonal antibodies. Thus, after acute ethanol administration to naïve rats (1 g/kg, i.p.), the reducing agent cyanoborohydride was injected into the lateral ventricle. Thereafter, the brain was dissected, homogenising separately the VTA from the rest of the brain. In parallel, brain and VTA homogenates from non-treated rats were incubated with different acetaldehyde concentrations (0-200 uM), in the presence of cyanoborohydride, in order to obtain a curve that relates acetaldehyde concentrations to adduct formation. Acetaldehyde was detected in both brain and VTA of rats treated with ethanol and cyanoborohydride. A linear correlation was found between the acetaldehyde concentration added to homogenates and the formation of adducts (r^2= 0.914 for total brain, r^2= 0.983 for the VTA), allowing to estimate brain and VTA acetaldehyde levels following acute ethanol administration.FONDECYT 1095021 (YI), 1110263 (PMR), 1120079 (MHM).

57.Statistical and conformational analysis of canonical and non-canonical base pairs in RNA and DNA structures. **Cares Galvez, J.**[1,2], Rodríguez, F.[1,2], Melo, F.[1,2]. Molecular Bioinformatics Laboratory, Millennium Institute on Immunology and Immunotherapy, [1], Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile.[2].

Canonical and non-canonical base pairs play a critical role in the stability of nucleic acids structure. They are also important to define function in RNA and DNA molecules. We have carried out an exhaustive analysis of the statistics and conformational variation for all possible canonical and non-canonical RNA base pairs (156 different base pair types in total) from a set of 268 non-redundant RNA structures. The most striking result is that the abundance of GC base pairs doubles that of AU pairs (48.3% versus 22.4%). This bias cannot be explained because of sequence composition issues of the RNA molecules. Additionally, high structural variation is observed for some particular base pair types, which cannot be explained simply by the number of inter-base hydrogen bonds.We have also extracted a total of 901 di-nucleotides and 773 tri-nucleotides substructures from a set consisting of 86 duplex DNA molecules at the Nucleic Acid Database (NDB) and analyzed the conformational variation of each type. Unexpectedly, some di-nucleotides and tri-nucleotide types rich in A and T are among those with the smallest structural variation. The data generated in this analysis will be useful for the prediction and assessment of three-dimensional structures of DNA and RNA molecules.This research was funded by grants from FONDECYT (1110400) and ICM (P09-016-F).

58.Interaction between BIRC5 a cancer protein with SMAC mimetic peptides, a fluorescence spectroscopy approach. **Carrasco, V.**[1], Guzmán, F.[1], Guzmán, L.[1], Aguilar, L.[1]. Pontificia Universidad Catolica de Valparaiso[1].

Finding new specific inhibitors for antiapoptotic proteins is of vital importance in order to develop new therapies against cancer. BIRC5 la member of IAP family (Inhibitor of Apoptosis Protein), is present in several kinds of cancer, and absent in normal cells. The rol of BIRC5 in survival of cancer cells is due to its interaction with the proapoptotic protein SMAC, a cell death inductor. Peptide mimetics to SMAC have been sugested and studied as possible IAP inhibitors and therefore as possible cáncer therapy drugs. In the present study peptides analogues to SMAC were synthesized and their interaction with BIRC5 was evaluated. Peptides were synthesized by Solid Phase Peptide Synthesis and later characterized by Circular Dichroism and Mass Spectrometry. BIRC5 was obtained using Molecular Biology techniques, being expressed in *E. coli* and later purified and characterized by Western Blot analysis and Circular Dichroism. The interaction between peptides and BIRC5 was studied using Fluorescence spectroscopy techniques.BIRC5 was obtained and identified by Western Blot analysis resulting in a band at 20 kDa. The mass spectrometry analysis of the peptides was in agreement with theoretical masses. Data provided by previous bioinformatics analysis was compared to experimental data obtained from fluorescence spectroscopy assays, providing a better insight into the peptides and BIRC5 interaction.Acknowledgements: This work was supported by Dirección de Investigación e Innovación of the Vicerrectoría de Investigación y Estudios Avanzados; project DII 037.427/2012.

59.Molecular function of glucokinase in hypotalamic glial cells. **Salgado, M.**[1], Tarifeño, E.[1], Llanos, P.[1], Yañez, M.J.[1], Villagra, M.[1], Martínez, F.[1], Uribe, E.[2], García-Robles, M.A.[1]. Laboratorio de Biología Celular, Facultad de Ciencias Biológicas, Universidad de Concepción[1], Laboratorio de Enzimología, Facultad de Ciencias Biológicas, Universidad de Concepción[2].

Glucokinase (GK) catalyzes the glucose phosphorylation with a high K_m value in different tissues involved in glucosensing. Previously, our group demonstrated localization of GK in the hypothalamic glial cells named tanycytes, suggesting that these cells play a role in the glucosensing system of the brain. In hypoglycemic conditions, hepatic GK is regulated at a post-translational level through binding to GK Regulatory Protein (GKRP), which functions as an anchor protein, modulating GK activity and mediating its nuclear translocation. Here we cloned and expressed GK and GKRP from tanycytes, the recombinant proteins were purified and the effect of GKRP on the activity and kinetic parameters of GK was studied. The kinetic characterization of GK showed a K_m value of ~10 mM for glucose. This value is according with reported Km for pancreatic and hepatic GK. GKRP showed a competitive inhibition over GK, with an IC_{50} of 0.29 uM and K_i of 0.13 uM. At protein level, we assessed the localization of GK and GKRP in liver and hypothalamus of rats under hypoglycemic, normoglycemic, and hyperglycemic conditions. Opposite to describe in liver, GK is localized at nuclear level in hyperglycemia, while in hypoglycemia is diffused into the citoplasm. We demonstrated that the tanycytes have a functional GK, which is inhibited by GKRP in vitro. Its dynamic localization in response to glucose in hypothalamus could indicate a new regulatory mechanism tissue specific.Grant support by FONDECYT 1100705, Center for Advanced Microscopy, CMA BIO BIO.

60.γ subunit of R-phycoerythrin from *Gracilaria chilensis*: sequence analysis and protein modelling. **Lobos, F.**[1], Martínez-Oyanedel, J.[1], Bunster, M.[1]. Laboratorio de Biofísica Molecular, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Casilla 160-C, Concepción, Chile[1].

Phycobilisomes are accessory light-harvesting complexes present in cyanobacteria and red algae, which are composed mainly by phycobiliproteins, polypeptides containing covalently attached chromophores known as phycobilins. The phycobilisomes from the red alga *Gracilaria chilensis* have three kinds of phycobiliproteins: R-phycoerythrin, R-phycocyanin and allophycocyanin. Besides, linker proteins associated with the phycobiliproteins are involved in the complex assembly and stabilization. However, in some particular cases, these linker proteins can also be involved in the light transfer process, as it is supposed to be the case of the γ subunit of R-phycoerythrin, which is a chromophorylated linker associated with the $(\alpha\beta)_6$ hexamer of R-phycoerythrin. So far it has not been possible to determine the structure of the γ subunit, and therefore the exact position within the R-phycoerythrin hexamer and the three-dimensional location of their chromophores is also unknown. Based on information available for other species, primers were designed and PCR reactions were performed using total DNA from *Gracilaria chilensis*. Using this PCR product, the gene for the γ subunit of R-phycoerythrin was sequenced and its protein sequence was deduced. This sequence was compared with γ subunits from other red algae and some features, such as intrinsic disorder prediction, signal peptide cleavage sites and bilin attachment sites were analyzed. Finally, a molecular model using protein threading is proposed. DIUC 211.037.012-1.0.

61.Sorbitol metabolism in *Arabidopsis thaliana*, a non-translocating sorbitol species. Parada, R.[1], Aguayo, M.F.[1], Zamudio, S.[1], Ampuero, D.[1], Handford, M.[1]. Laboratorio de Biología Molecular Vegetal, Facultad de Ciencias, Universidad de Chile[1].
Sorbitol is the main photosynthesis product in *Rosaceae* species (apples, peaches). This sugar alcohol is produced in source organs by the action of sorbitol 6-phosphate dehydrogenase, A6PR and phloem-transported to sink organs where, by the action of sorbitol dehydrogenase (SDH) it is oxidised to fructose, which can be either stored or metabolised. On the other hand, in almost all other plant families, including *Brassicaceae*, sucrose is the principle sugar transported. Nevertheless, sorbitol and/or SDH activity have been detected in some of these families, but their physiological roles have yet to be understood. As an approach to determine the roles of sorbitol and related enzymes in non-sorbitol translocating species, we have identified the putative key enzymes involved in sorbitol metabolism in *Arabidopsis thaliana*, and named them AtA6PR and AtSDL. Both enzymes share high amino acid identity with their characterised homologs from apple (70% and 77%, respectively). We have shown that both *Arabidopsis* proteins are localised in the cytosol. Here, we demonstrate that AtSDL is differentially-expressed in different organs, and that. recombinant His-AtA6PR and His-AtSDL produced *in vitro* are capable of forming and oxidising sorbitol, respectively. Additionally, *atsdl-* mutants show enhanced resistance to dehydration stress. Finally, in order to determine the *in vivo* role of sorbitol in *Arabidopsis*, we are over-expressing AtA6PR in wild type and *atsdl-* plants, and will detect the levels of the respective proteins using polyclonal antisera. Funding: Fondecyt 1100129, CONICYT Magister 22110701 (Francisca Aguayo) and 22100522 (Diego Ampuero).

62.*Helicobacter pylori* induced loss of survivin expression and gastric cell viability is linked to secretion of bacterial gamma glutamyl transpeptidase. Valenzuela, M.[1,2], Bravo, D.[1], **Canales, J.**[1], Toledo, H.[2], Quest, A.F.[1]. Centro de Estudios Moleculares de la Célula (CEMC), Facultad de Medicina, Universidad de Chile.[1], Laboratorio de Microbiología Molecular, Programa de Biología Celular y Molecular, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile.[2].
Introduction: *Helicobacter pylori (H. pylori)* is a human pathogen that causes gastric disorders and adenocarcinoma. The development of gastric cancer is associated with oxidative stress promoted, in part, by bacterial factors. *H. pylori* gamma glutamyl transpeptidase activity (GGT) has emerged as a novel virulence factor associated with the generation of reactive oxygen species (ROS) and apoptosis. Previously, we demonstrated that *H. pylori* induces survivin down-regulation and apoptosis in gastric cells. Here we evaluated whether these effects are mediated by *H. pylori* GGT. **Methods:** Gastric cancer cell lines MKN45 and AGS were exposed to *H. pylori* wild type bacteria, GGT-deficient mutant bacteria or to their respective culture supernatants. Survivin expression was measured by western blotting and cell viability using MTS assays. **Results:** The *H. pylori* wild type strain and its culture supernatants induced loss of survivin expression and gastric cell viability. Effects observed with the GGT-deficient mutant bacteria or its culture supernatants were significantly diminished. *H. pylori*-induced survivin down-regulation was blocked in the presence of the iron chelators Tiron or Deferoxamine, but not by the antioxidant Trolox. **Conclusions:** These results identify GGT as a *H. pylori* virulence factor that is required to trigger Fe+2-dependent loss of survivin and gastric cell viability. Moreover, the data suggest that accumulation of GGT activity in the medium is sufficient to promote gastric cell death in vitro. **Acknowledgements.** FONDAP 15010006, FONDECYT 1090071, ACT1111 (AFGQ), FONDECYT 1120126 (HT), CONICYT Student Fellowship (JC).

63.Caveolin-1 amino acid Ser80 is crucial for tumor suppression in a melanoma model. Díaz, M.I.[1,2], **Ávalos, Y.**[1], Sanhueza, C.[1], Ortiz, R.[1], Hetz, C.[2], Quest, A.F.[1]. Laboratorio de Comunicaciones Celulares, Centro FONDAP de Estudios Moleculares de la Célula (CEMC), Facultad de Medicina, Universidad de Chile[1], Laboratorio de Estrés celular y Biomedicina, Centro FONDAP de Estudios Moleculares de la Célula (CEMC), Facultad de Medicina, Universidad de Chile[2].
Introduction: The scaffolding protein Caveolin-1(Cav-1) functions as a tumor suppressor, often via binding to and inhibition of target proteins. Previous results from our laboratory showed that the tumor adaptive unfolded protein response (UPR) is inhibited by Cav-1. To date, no mutations in Cav-1 have been associated with function as a tumor suppressor and UPR regulation.**Methods:** C57BL6 mice were subcutaneously injected with either mock-transfected B16F10 melanoma cells or cells expressing Cav-1(wt), Cav-1(S80A) or Cav-1(S80E). After 15 days, tumors were extracted and UPR markers were evaluated. For in vitro experiments, the same cells were treated either with tunicamycin or subjected to hypoxia for 24 h. Changes in UPR markers were evaluated by PCR and Western blotting. Co-localization of Cav-1 with an endoplasmic reticulum (ER) marker was determined by indirect immunofluorescence analysis. **Results:** Tumor suppression due to expression of Cav-1(wt) was not observed upon introducing Cav-1(S80A) or Cav-1(S80E) into B16F10 cells. Induction of UPR markers was diminished in B16F10 cells expressing Cav-1(wt) exposed to tunicamycin or hypoxia. Interestingly, however, UPR responses were also attenuated in cells expressing the Cav-1 mutants, although like Cav-1(wt), Cav-1(S80A) and Cav-1(S80E) co-localized to a similar extent with ER markers following tunicamycin treatment or exposure to hypoxia. Thus, Ser80 is crucial in this experimental model for Cav-1 function as a tumor suppressor but does not appear to be relevant to UPR inhibition. **Acknowledgements:** FONDAP 15010006 (AFGQ), FONDECYT 1090071 (AFGQ, CH), ICEGB CRP/CH 108-03 (CH, AFGQ), CONICYT (MID, YA).

64.Supported choice of clustering algorithms and partitions in hierarchical clustering of biological data. Slater, A.W.[1,2], Norambuena, T.[1,2], Melo, F.[1,2]. Molecular Bioinformatics Laboratory, Millennium Institute on Immunology and Immunotherapy.[1], Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile.[2].
A classic problem in biology is to organize entities through a hierarchical classification system. Such a classification system is represented in the graphical form of a tree, which is constructed with a hierarchical clustering algorithm that takes as input the previously measured distances among all entities. There are two fundamental choices that need to be defined in this process. First, the selection of a specific hierarchical clustering algorithm, and second the definition of a particular distance-cutoff that provides the total number of groups and the list of representative cluster members. Both choices are usually not supported by formal criteria when hierarchical clustering of biological data is presented.In this work, we address the problem of implementing a formal procedure that allows, for any given dataset, to extract: a) the best suited hierarchical clustering algorithm; b) a specific distance-cutoff value that generates a partition of the tree into different groups, where intra and inter group similarities are maximized and minimized, respectively, and c) a list of representative members for each generated group, which have the minimal average distance against all members belonging to the same group. We provide several examples from real tasks of biological data clustering to illustrate the usefulness of this method. This software tool can assist the researcher in the analysis of biological data clustering with a formal criteria rather than the usual practice where an arbitrary selection of both the clustering algorithm and the distance-cutoff value is made.ACKNOWLEDGEMENTS:FONDECYT_1110400&ICM_Nº_P09-016-F.

65.Use of HEK293T cells to evaluate Infectious Salmon Anemia Virus fusion protein activity. **Castillo-Jara, S.**[1,2], López, X.[3], Spencer, E.[2,3], Cortez-San Martin, M.[2,3], Montoya, M.[2,3], Acuña-Castillo, C.[2,3]. Universidad de la Frontera[1], Centro de Biotecnología Acuícola, Universidad de Santiago de Chile [2], Departamento Biología, Universidad de Santiago de Chile[3].

ISA virus is an enveloped virus member of Orthomyxoviridae family. Its genome is RNA simple strand negative composed of 8 segments, where the segment 5 of 1368 nucleotides encoding the viral fusion protein (F). We have characterized a variant of the virus which has an insertion in the segment 5 (IN4) that add 33 nucleotides. Our goal is to determine whether this variant changes the capacity to induce cellular fusion. The segment 5 was cloned into on pIres2-EGFP. Then, HEK293 cells were transfected with this plasmid and were obtained stable clones. Cellular fusion was evaluated by conventional and fluorescent microscopy using cell fluorescents probes. We use pH variation to evaluate fusion activity to demonstrate pH-dependence fusion in HEK cell model. Using conventional-microscopy was looked that the fusion protein was able to induce formation of cells syncytium. In our experiments we looked fusion events at pH lower than 7,5 and under trypsine pretreatment, similar to described previously on *Salmo salar* cellular line. The results validate HEK293 as a model study of fusion for F as well as the use of a protein derived from virus of salmonids for induce cellular fusion in human cells, in agreement with our preliminary results, both variants of F appears to be differential activity. Fondecyt 1110734, Fondecyt 11110212 and InnovaCorfo 09MCFSS66-98.

66.Reconstruction of the last common ancestor of the bifuncional HMPK/PLK and of the specific HMPK enzymes of ATP-dependent coenzyme kinase family. **Bravo-Moraga, F.**[1], Castro-Fernández, V.[1], Ramírez-Sarmiento, C.[1], Guixé, V.[1]. Laboratorio de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Chile.[1].

The ATP-dependent coenzymes kinase family belongs to the ribokinase superfamily, where members with kinase specificity for hydroxymethylpirimidine (HMPK) and pyridoxal (PLK) were found. An interesting feature in this family is the existence of a bifuncional enzyme from *Bacillus subtilis* that can phosphorylate both substrates. At date, it is not known if this bifunctionality is an ancestral trait or an evolutionary novelty in this family. In this work we have reconstructed the phylogenetic tree of the family which clearly show two separate groups with different activities; bifuncional HMPK/PLK and specific PLK. Using Bayesian method we inferred the sequence of the last common ancestor of both activities. With this sequence we built a homology model which keeps the general characteristics of the family, including residues of conserved motifs essential for catalysis, like GXGC and NXXE motifs, and the loop of the active site that covers the substrate. Also, we performed docking trials to evaluate the interaction of pyridoxal and hydroxymethylpyrimidine substrates at the active site of the ancestral enzyme. Both substrates have negative interaction energies and the hydroxyl that is phosphorylated is directed toward the catalytic cysteine and ATP, in a geometry that would allow the transfer reaction. Our results indicate that this ancestral enzyme could have been functional. Fondecyt-1110137.

67.3D-pMHC: a curated database of peptide-MHC complex three-dimensional structures. **Gutierrez, F.**[1,2], Melo, F.[1,2]. Molecular Bioinformatics Laboratory, Millennium Institute on Immunology and Immunotherapy[1], Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile[2].

The major histocompatibility complex (MHC) molecules play a key role in the immune system. When a pathogen infects the cell, pathogenic proteins are processed by the antigen processing machinery into peptides and only a small fraction is loaded onto the MHC molecules. The peptide-MHC complexes (pMHC) are expressed on the cell surface and the immune response is elicited via T-cell receptor (TCR) binding. The understanding of the structural principles involved in the selection of specific antigenic peptides by the different MHC alleles and TCR/pMHC recognition is critical in drug design. 3D-pMHC is a curated database of peptide-MHC class I and II complexes containing structures of 367 complexes with a resolution <= 3.5 ?. To create the database, the complete Protein Data Bank was screened with TopSearch, a fast structure comparison software tool. Afterwards, from each structure, the pMHC complex was extracted and the protein chains renamed in a standardized way. Structures lacking the peptide or peptides with missing residues, fusion proteins, MHCs bound to non-classical ligands and other unrelated proteins similar in structure to the MHCs were discarded from the database. Finally, an analysis of redundancy was carried out, thus generating a database that contains only unique entries. The aim of building the database is to enhance the understanding of the binding mechanism of the pMHC complexes, in order to obtain information with predictive value for future development of new vaccines.This work was funded by grant P09/016-F from ICM.

68.Structural features of the NAD(P) binding sites in the SCOP Folds. **Fuentealba, M.**[1], Cabrera, R.[1]. Laboratorio de Bioquímica y Biología Molecular, Departamento de Biología, Facultad de Ciencias, Universidad de Chile[1].

The nicotinamide adenine dinucleotides, NAD and NADP, are cofactors commonly used by enzymes that catalyze biological redox and non-redox reactions. Despite the structural similarity of this cofactors, the NAD(P) binding proteins display an impressive power of molecular recognition, since they are capable of discriminate by the presence of the phosphate group. In this sense a better understanding of the recognition determinants could be drawn through the analysis of the whole repertory of known structures. In this work a non-redundant set of high-resolution structures of NAD(P) binding proteins was selected from the protein structures in the PDB database. Only extended conformations of the NAD(P) were further analyzed. Each structure was classified according to the SCOP fold and family criteria, and the subset of binding site residues was structurally superposed for each family based on the point clouds method (MultiBind). The superposed residues were aligned to specify the conserved NAD(P) binding motif for each family and were mapped on the structure of a representative family member. Using the Amber force field in explicit solvent, molecular dynamics simulations of these representatives were performed, and the NAD(P) binding pockets were energetically analyzed. We observed fold-independent trends for the residues lining the binding pocket that allows specific recognition, such as the presence of a negative charge in NAD binding proteins and a mono or dual positive charge in the NADP binding proteins, providing high ligand interaction energies. FONDECYT 1121170.

69.Tellurite-induced oxidative damage affects *Escherichia coli* NADH dehydrogenase I and II activities in aerobic conditions. **Díaz Vásquez, W.A.**[1], Abarca Lagunas, M.J.[1], Cornejo Leiva, F.A.[1], Pinto Bizama, C.A.[1], Vásquez Guzmán, C.C.[1]. Universidad de Santiago de Chile[1].

Tellurite is toxic for most Gram-negative bacteria. *Escherichia coli* exposed to sublethal concentrations of tellurite suffers oxidative stress showing decreased cellular thiols content, accumulation of metabolites such as pyruvate, Alpha-ketoglutarate, phosphoenolpyruvate, and increased Reactive Oxygen Species levels (ROS). Some metabolic enzymes showed loss of activity in tellurite-exposed cells. In this work tellurite effect on NADH dehydrogenase I and II components (NDH-I and NDH-II) of the Electron Transport Chain (ETC) was analyzed. The ETC reduces oxygen to water using NADH and/or $FADH_2$, generating a proton motive force.*E. coli* wild type and NDH-I-and NDH-II mutant derivatives were exposed to sublethal tellurite concentrations to determine growth curves, ROS and superoxide generation and oxygen consumption. We determined the level of *nuoF*, *ndh*, and other transcripts of respiration related genes by qPCR. Membranes were assayed for NADH / dNADH dehydrogenase and cytochrome oxidase activities, and protein carbonylation was also determined. Our results showed a reduced growth and oxygen consumption especially in the NDH-I$^-$ strain and increased levels of ROS, particularly superoxide. Transcripts analysis showed an induction of oxidative stress response genes, anaerobiosis, respiration regulators, and ndh. Membranes showed loss of NDH-I activity and proteins carbonylation, especially in NDH-I$^-$ cells.We conclude that tellurite damage to membranes is mainly oxidative, inactivating NDH-I and not NDH-II dehydrogenase. The inability of bacteria to breath results in the induction of *ndh* and other anaerobics genes. Funding: FONDECYT 1090097, CONICYT, Scholarships Doctoral Thesis Support 24121087, DICYT and VRID USACH.

70.Development of immunoassay for pathogens detection using fluorescence polarization. **Silva, V.**[1], Cuevas, F.[2], Aguilar, L.F.[1]. Pontificia Universidad Católica de Valparaíso[1], Universidad Santo Tomas[2]. Sponsored by Guzmán, L.

Currently there are many commercial kits capable of detecting effectively the antibody antigen ratio, however many of them lack the capability to detect such interaction when working with small amounts of sample. To be able to improve the sensitivity of immunological techniques is of vital importance, in order to detect even smaller quantities of antigen antibody complex. In the aim to improve sensitivity to such kits, fluorescence spectroscopy techniques have been incorporated. Even though using fluorescence methods has shown a significant improvement of sensitivity, such figures of merits can be improved even more, providing the possibility to work with lesser quantities of antibody/antigen complex and also providing greater specificity. Using polarized fluorescence spectroscopy also provides greater specificity to the analysis. The aim of this work is to develop a methodology based on fluorescence polarization techniques in order to more effectively detect the presence of pathogens. The study model used for this work consisted in serum of specimens infected with pathogens to guarantee the presence of antibody and antigen in the sample. The control group consisted in uninfected specimens. The antigen sample was incubated with fluorescent probe allowing the covalent junction between antigen and probe and fluorescence polarization was measured. As result a strong correlation between presence of infection and increased polarization was observed, showing the sensitivity in comparison to a commercial kit.Acknowledgements: Doctoral Felowship from CONICYT is gratefully acknowledged by VS.

71.Molecular modeling and structural analysis of K2P channels interacting with the inhibitor A1899. **Martínez, G.**[1], Alzate, J.[1], González, W.[1]. Centro de Bioinformática y Simulación Molecular (CBSM), Universidad de Talca[1].

Two-pore-domain potassium channels (K2P) are selective ion channels, characterized by a 4 transmembrane domain (M1-M4) topology and 2 pore-forming domains per subunit. Some of the most studied roles of K2P channels include the maintenance of the resting potential, regulation of the excitability of the cells, ion transport and metabolic regulation.A major difficulty to understand the role of these channels as therapeutic targets is the lack of selective pharmacology. It is necessary to understand how the therapeutic agents act and modify the structure of K2P channels to develop specific drugs. To date only the molecule A1899 has shown high selectivity for different K2P channels. The residues involved in the formation of the drug binding site of one K2P channel (TASK-1) have been identified, nevertheless, the molecular mechanism of A1899 to bind to these channels is unknown. These data were taken as basis for determining the specific binding site for A1899 in K2P channels. Using docking simulations we found the most stable complexes between receptor?s binding site and the inhibitor. Subsequently, molecular dynamics of receptor-ligand were performed to equilibrate the complexes achieved by docking. The results achieved describe the relationship between A1899 and K2P channels, and suggest how this molecule blocks each channel differently, showing good agreement with electrophysiological data.Although the results agree with biological information available, further analyses are required to support the findings and establish the individual contribution of each residue involved in the K2P channel block.

72.Comparative modeling of the TraY DNA binding protein reveal a trefoil knot topology. **Molina, J.**[1], Floor, M.[1], Reyes, J.[1], Bustamante, A.[1], Baez, M.[1]. Laboratorio de Bioquímica, Departamento de Bioquímica y Biología molecular. Facultad de Ciencias Químicas y Farmacéuticas. Universidad de Chile. [1]. Sponsored by Cabrera, R.

Protein knots are intriguing topologies that have been gradually populating the Protein Data Bank, and are now being recognized as significant structural motifs. The existence of knots in proteins is challenging in both experimental and theoretical settings.The Ribbon-Helix-Helix (RHH) family of transcription factors present a conserved structure of four helices flanked by a two-stranded antiparallel beta-sheet. While typical RHH proteins are homodimers created by association of two RHH motifs, there are a few monomeric proteins in this family. TraY is a site-specific DNA binding protein with a role in the initiation of conjugative DNA-strand transfer that sequence shows a duplicated RHH motif contained into a single polypeptide chain. Each RHH sequence motif of TraY was aligned using a structure-based alignment of ten homodimeric RHH structures to create a comparative model. The model shows a trefoil knot formed by a loop that connects the first RHH motif and the starting beta strand from the second RHH motif, although a segment at the C-terminal end of TraY cannot be modeled. Due to the low percent of homology and small size of the RHH proteins, biochemical experiments are in progress to validate the TraY model. So far, these results support the hypothesis that protein knots might have evolved by a mechanism of gene duplication and fusion from an ancestral dimer and give a new model to study the folding mechanism of proteins with knotted topologies. Fondecyt 11110534.

73.Similarities of binding sites between monoaminergic proteins and nicotinic acetylcholine receptors (nAChRs) to the rational design of promiscuous drugs. Möller Acuña, P.[1,2], Reyes Parada, M.[1]. Universidad de Santiago de Chile[1], Universidad de Talca[2]. Sponsored by González, W.

Psychiatric disorders have been growing steadily, however due to the complexity of the neural circuits that regulate them have been limited their physiopathological understanding. The finding of a suitable solution against these diseases is a hard work, because it involves changes in neuronal connection network where the monoaminergic system and the neuronal nicotinic acetylcholine receptors (nAChRs) play an important role. Considering that the psychiatric disorders are the result of a complex network of molecular events, it is essential to develop drugs that act in a polyselective way. In this work we built molecular models of a monoaminergic family proteins (NET, SERT, MAO-A and MAO-B) and nAChRs (alpha3, alpha5, alpha5-beta4), which are directly implicated in neuronal disorders. Structural similarity analyses were performed to compare the binding site of these proteins and molecular docking simulations were carried out to evaluate the affinity of amphetamine derivatives in the binding pockets. The most optimal complexes were simulated by molecular dynamic to characterize the specific interactions that regulate the inhibitors selectivity. Our results show a high structural similarity between the different proteins indentifying conserved residues in their active sites. In addition, structural analyses show that the bind of amphetamine derivatives ligands is mediated by hydrogen bonds, electrostatic and phi-phi interactions which determining their selectivity. This information is envisioned to prove useful for the design of novel neuronal inhibitors. Ack:FONDECYT N? 1090037, FONDECYT N? 11085002.

74.Characterization of the role of residue Y671 in the ionic conductance of the TRPV1 channel. Vásquez, Y.[1], Poblete, H.[1], Brauchi, S.[2], González-Nilo, D.[3]. Centro de Bioinformática y Simulación Molecular, Universidad de Talca.[1], Centro Interdisciplinario de Neurociencias de Valparaiso, Facultad de Ciencias, Universidad de Valparaiso.[2], Centro de Bioinformática y Biología Integrativa, Universidad Andrés Bello.[3]. Sponsored by González, W.

The transient receptor potential V1 (TRPV1) channel, also known as the capsaicin receptor, is a non-selective cation channel with a high relative Ca2+ permeability. This channel is predominantly expressed in sensory neurons activated by temperature (> 42C), low pH (< 6) and ligands. TRPV1 is responsible for the perception of noxious stimuli in sensory neurons and, in particular, for the perception of pain. Thus, it has been proposed as a potential target for analgesic drugs. The narrowest portion of the pore, known as the selectivity filter, has been widely studied for its effect on the conductance of the channel; however, additional research has revealed other groups of conserved amino acids that modulate the conductance in TRPV1 channels. Specifically, mutations of the Y671 residue by amino acids with different physicochemical properties alter the conductance as well as the characteristic ability of the TRPV1 channel to become desensitized due to overstimulation. Therefore, with the purpose of characterizing the structural and energetic effects of mutations of this residue, we constructed molecular models of TRPV1 and several mutants identified in experiments. HOLE analyses and PMF/Poisson-Boltzmann calculations were performed to evaluate changes in the pore structure and relative affinity for K+ ions due to the mutations. Our results show that negatively charged residues at position 671 increase the ion permeability, while large or positively charged residues result in a large energetic barrier to ion passage. ACK : CINV-ICM.

75.Binding of K+ to phosphofructokinase-2 enhances the ATP allosteric inhibition and dimer-tetramer transition induced by the nucleotide, but these two phenomena are unrelated. Vallejos, G.[1], Villalobos, P.[1], Blanco, A.[1], Baez , M.[2], Babul, J.[1]. Departamento de Biología, Facultad de Ciencias, Universidad de Chile.[1], Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.[2].

Phosphofructokinase-2 (Pfk-2) from E. coli catalyzes the MgATP dependent phosphorylation of fructose-6-P to fructose-1,6-bisP. The enzymatic activity of Pfk-2 is inhibited upon allosteric binding of MgATP, which is enhanced by specific binding of monovalent cations such as K+. Moreover, Pfk-2 is a homodimer able to form homotetramers upon MgATP binding. In this work we explore the relationships between K+ binding and the dimer-tetramer transition through kinetic assays, MgATP binding experiments and limited proteolysis using the wild type enzyme and mutants unable to form tetramers. To understand the problem at atomic level, we also performed molecular dynamics simulations. Kinetic measurements show that the presence of K+ enhances the MgATP inhibition independently of the dimer-tetramer transition. The sigmoidicity of the saturation curves and the apparent affinity for MgATP for the wild-type enzyme was increased by the presence of K+, while the mutant did not show variations on these parameters. Furthermore, limited proteolysis shows that the MgATP-dimer complex is partially protected against trypsin cleavage, protection which is enhanced upon tetramer formation. Moreover, the presence of K+ increases the MgATP induced protection in both the dimeric and tetrameric conformations. Additionally, molecular dynamics simulations show that K+ causes changes in the flexibility of important residues for the dimer-dimer interface in tetrameric Pfk-2.These results suggest that the binding of K+ favors the dimer-tetramer transition induced by MgATP and separately enhances the allosteric inhibition produced by the nucleotide in the dimeric enzyme. Fondecyt 1090336.

76.FragProt, a webserver and database of protein fragments clustered by structural similarity. Rodríguez, F.[1,2], Slater, A.[1,2], Melo, F.[1,2]. Molecular Bioinformatics Laboratory, Millennium Institute on Immunology and Immunotherapy[1], Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile[2].

Protein fragments have been, for a long time, an important research subject in bioinformatics, with applications in protein homology modeling, secondary and tertiary structure prediction, study of sequence-structure relationships and drug design. Here we present an upgrade of our webserver FragProt, a database of protein-derived fragments clustered by structural similarity. These fragments are extracted from different protein datasets, which include a large number of peptides from a non-redundant set of monomeric and globular proteins (MGP), a non-redundant dataset of protein-DNA complex interfaces (PDIdb) (1), a database of protein-DNA complexes (PDC), and a non-redundant set of trans-membrane (TM) proteins, among others. Approximately 60,000, 14,000 and 18,000 fragments were extracted from TM, PDIdb and MGP datasets, generating a total of about 16,000, 6,000 and 8,000 independent structural clusters, respectively. Sequence conservation, secondary structure and solvent accessibility surface area were calculated for every fragment in the context of the native structure. Finally, a structure based search method was implemented using the Ultrafast Shape Recognition (USR) algorithm (2), allowing the user to provide a fragment in PDB format as a query to search in the database. These improvements, in addition to other features like different Jmol visualizations and dynamic searches, make this database a useful tool to explore the growing universe of peptide conformations and to study the sequence-structure-function relationships in proteins. **Acknowledgements:** FONDECYT (1110400) and ICM (P09-016-F). **References** 1. *BMC Bioinformatics. 2010*, 11:262. 2. *J Comput Chem.* 2007 Jul 30;28 (10):1711-23.

77.Role of lysine 27 in the MgATP inhibition of phosphofructokinase-2 from E. coli. **Soto, F.A.**[1,2], Villalobos, P.[1], Baez, M.[3], Babul, J.[1]. Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile.[1], Instituto de Química, Pontificia Universidad Católica de Valparaíso.[2], Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.[3].

Phosphofructokinase-2 (Pfk-2) is a member of the Ribokinase family that catalyzes the transfer of the γ-phosphate from ATP to fructose-6-P. Differently from other sugar kinases of the family, Pfk-2 shows allosteric inhibition by MgATP. It has been suggested that the inhibition is required to avoid a futile consumption of ATP in E. coli. The MgATP inhibition is coherent with the presence of a second MgATP binding site localized close to active site. Comparison of several crystallographic structures of Pfk-2 obtained in presence of MgATP, fructose-6-P or both substrates suggests a critical role of Lys27 for the allosteric regulation and also for the active site catalytic mechanism. Interestingly, the Lys27 side chain is sequestered alternatively by the phosphate moieties of both substrates; the ligand (ATP or fructose-6-P) that binds first to the active site would diminish the affinity for the second one. The change of Lys27 by glutamine or valine decreases the kcat value 50 times and renders the enzyme insensitive toward the allosteric inhibition induced by MgATP. However, intrinsic fluorescence measurements show that thereare no changes on the binding parameters of the substrates with respect to the wild type enzyme. These results suggest that Lys27 stabilizes the transition state reducing the negative charges in the phosphoryl group that is transferred during the nucleophilic attack and can be considered a key residue for the MgATP allosteric inhibition of this enzyme.(Fondecyt 1090336).

78.Structure determination in the crystalline state and in solution reveals a conserved mechanism for catalysis in hiperthermophilic ADP-dependent glucokinases. **Herrera-Morandé, A.**[1,2], Rivas-Pardo, J.A.[1], Castro-Fernández, V.[1], Fernández, F.J.[2], Vega, M.[2], Guixé, V.[1]. Departamento de Biología, Facultad de Ciencias, Universidad de Chile [1], Centro de Investigaciones Biológicas-CSIC, Madrid, España[2].

ADP-dependent glucokinases (GKs) represent a unique family of kinases that belong to the ribokinase superfamily. In the hyperthermophilic archeon Thermococcus litoralis, the ADP-dependent GK (TlGK) phosphorylates D-glucose in the first step of a modified Embden-Meyerhof pathway. In this work we evaluate and compare the conformational changes observed in the crystal structures with those determined in solution, in order to understand their interplay with the GK function. To achieve this goal we employed an integrated approach including enzyme kinetics, SAXS, X-ray crystallography and phylogenetic analysis. The enzyme presents a sequential ordered mechanism ordered mechanism in which Mg-ADP is the first substrate to add to the enzyme and Mg-AMP the last product to be released. SAXS data indicate that the compactness of the enzyme changes following the sequential events of binding of substrates. The high-resolution crystal structures of TlGK in the apo form and as a ternary complex with Mg-ADPβS-D-glucose shows a two domains organization, and allow us to define the extent of the conformational changes. Our results provide insights into determinants of the structural changes that accompany ligand binding both in solution and in the crystalline state. Detailed inspection of clusters of residues involved in the open-closed conformational transition and the quest for them in the phylogenetic context of the ADP-dependent kinases family, leads us to propose a general mechanism for this conformational transition conserved throughout this family. Fondecyt 1110137, Spanish Ministry of Science and Innovation grants PET2008_0101, BIO2009-11184 and BFU2010-22260-C02-02.

79.Identification of the binding site of phosphatidylinositol-bisphosphate (PIP2) in the transient receptor potential vanilloid type 1 channel (TRPV1). **Poblete, H.**[1], Oyarzun, I.[2], Comer, J.[3], Vásquez, Y.[1], Latorre, R.[2], González-Nilo, D.[3]. Centro de Bioinformática y Simulación Molecular. Universidad de Talca.[1], Centro Interdisciplinario de Neurociencias de Valparaiiso, Facultad de Ciencias, Universidad de Valparaiso.[2], Centro de Bioinformática y Biología Integrativa. Universidad Andrés Bello.[3].

Phosphatidylinositol 4,5-bisphosphate (PIP2) plays a central role in the activation of several transient receptor potential (TRP) channels. Specifically, the TRPV1 channel is a polymodal receptor activated by a range of stimuli including temperature, capsaicin, extracellular acidification, and lipids such as PIP2. The channel PiP2 binding site has not been explored in detail; however, a pocket called the TRP box has been postulated, located in the proximal region of the C-terminus, which is highly conserved in the subgroup of the Thermo-TRP channels. This region has been suggested as a structural motif that binds PIP2, stimulating TRPV1 channel activation, which in turn is responsible for the perception of pain and has been implicated as a causative factor in inflammation-related heat hyperalgesia. With the purpose of identifying the most probable PIP2 binding site in the channel, we constructed a molecular model of TRPV1 and performed coarse-grain molecular dynamics simulations, which revealed the favorable position of the channel in the membrane and the spatial distribution of PIP2 on the microsecond timescale. Furthermore, docking and molecular dynamics simulations were performed to characterize, at molecular level, the specific interactions between PIP2 and the TRP box, permitting the identification of the residues involved in the binding of this phospholipid. Acknowledgments: Beca doctoral CONICYT, Centro Interdisciplinario de Neurociencia de Valparaiiso that is a Millennium Science Institute (CINV-ICM).

80.Effects of monovalent cations on the allosteric MgATP inhibition of phosphofructokinase-2 from *Escherichia coli*. **Blanco, A.**[1,2], Villalobos, P.[1], Baez, M.[3], Babul, J.[1]. Departamento de Biología, Facultad de Ciencias, Universidad de Chile.[1], Escuela de Ingeniería en Bioinformática, Universidad de Talca.[2], Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.[3].

Phosphofructokinase-2 (Pfk-2) belongs to the ribokinase family, being the only member with an allosteric site for MgATP (MgATPa), an inhibitor of the enzymatic activity. Moreover, biochemical studies show that K^+ (but not Na^+) enhances the allosteric inhibition, increasing the affinity for MgATPa, without alteration of the catalytic constants and substrates affinities. In agreement with this, the three-dimensional structure of Pfk-2 with K^+ or Cs^+ shows a monovalent cation-binding site. This site is highly conserved in the ribokinase family members and in Pfk-2 is adjacent to the MgATPa binding site. The presence of either K^+ or Cs^+ does not induce large conformational changes to the enzyme. The aim of this work is to understand the molecular mechanism through which K^+ increases the affinity for MgATPa and the structural aspects related to the selectivity of the monovalent cation-binding site using classical Molecular Dynamics (MD) and Free Energy Perturbation (FEP) simulations. Comparisons between MD of Pfk-2 with and without K^+, show distances closer to the experimental values (~3.5 Angström) for the π-stacking interaction between Tyr23, whose side chain makes a crucial interaction for the inhibition, and the adenine ring of MgATPa when K^+ is present. Furthermore, FEP calculations indicate that each monovalent cation-binding site in Pfk-2 has a preference for K^+ relative to Na^+ of about 3 kcal/mol. These results are in agreement with the effect and the selectivity of monovalents cations observed on the kinetics properties of Pfk-2. Fondecyt 1090336.

81.NXXE and GXGD motifs play an important role in the activity and regulation of human ribokinase. **Quiroga, D.**[1], Guixé, V.[1]. Laboratorio de Bioquímica y Biología Molecular,Facultad de Ciencias, Universidad de Chile. [1].

Ribokinase (RK) belongs to the ribokinase superfamily and catalyzes the phosphorylation of D-ribose. Even though ribose acts in important metabolic steps, kinetic studies of human RK are scarce and preliminary. In the ribokinase superfamily there are two conserved motifs, NXXE and GXGD, localized at the active site of its members. The GXGD motif contains an aspartic residue propose to act as the catalytic base, while the NXXE motif is related with the proper use of Mg^{+2} and PO_4^{-3} ions. We study the role of residues N199, E202 and D269 of the NXXE and GXGD motifs respectively, on the enzymatic activity and PO_4^{-3} regulation on the human ribokinase enzyme. The N199L mutant presents a k_{cat} which is 980 times lower than the one determined for the wild type enzyme, while the K_m value for D-ribose decreases 2 fold and for MgATP increases 44 fold. The E202L mutant has a 280 fold fall in the k_{cat} value, while the K_m value for the sugar diminish 2 fold and for MgATP increases 37 fold. D269N mutant also show a great decreased in the catalytic activity. Although high PO_4^{-3} concentrations increase the activity of wild type RK, the activity of the three mutants was not altered in the presence of this ion. These results suggest that the N199, E202 and D269 residues play an important role in binding of substrates and catalysis, as well as in PO_4^{-3} regulation. (Fondecyt 1110137).

82.Estimation of binding free energy of antagonists against different subtypes of nAChRs: effect of clustering in the application of the MM-GBSA method. **Pareja, C.**[1], Adasme, F.[1], Iturriaga-Vásquez, P.[2], Alzate-Morales, J.[1]. Centro de Bioinformática y Simulación Molecular (CBSM), Escuela de Ingeniería, Universidad de Talca, 2 Norte 685, Casilla 721, Talca, Chile.[1], Laboratorio de Química Biodinámica, Departamento de Química, Facultad de Ciencias, Universidad de Chile, Las Palmeras 3425, Santiago, Chile[2]. Sponsored by González, W.

Nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels, which are present mainly in the central nervous system cells. It is known that nAChRs are related to several neurological disorders such as Alzheimer's disease, Parkinson's, Schizophrenia, anxiety, depression as well as nicotine addiction.Its binding site is composed of highly conserved aromatic amino acids and hydrophobic residues (aromatic box), where the binding behavior of several ligands has been reported. Generally, the cationic head of various ligands locates near to this box and to the aromatic side chain of a specific Trp residue, establishing a cation-pi interaction and several hydrogen bonds with neighboring residues.Considering the interactions described above, a free binding energy calculation protocol based on the MM-GBSA method will be applied to a series of ligands against to the alpha4(2) beta2(3), alpha4(3)beta2(2) and alpha4(2)alpha5(1)beta2(2) nAChR subtypes. To include the dynamic behavior of the binding, representative structure clusters will be selected from molecular dynamics simulations using a technique that relies on intermolecular interaction information. We expect to find a correlation between the biological experimental data (IC50) of several known ligands against nAChRs and the binding free energy (deltaGbind) calculated computationally for the corresponding ligand-receptor complexes. This information will be useful for designing new drugs that will be effective against various nAChR-related diseases. Acknowledgements:J.A.M. and C.P.B. acknowledge the financial support through project FONDECYT No 11100177. F.A. thanks a doctoral fellowship awarded from Government of Chile through CONICYT.

83.Energetic contribution of K18 and R50 to the 2' phosphate moiety measured from the term k_{cat}/K_M of wild-type and mutant forms of the glucose 6-phosphate dehydrogenase from *Escherichia coli*. **Muñoz, R.**[1], Cabrera, R.[1]. Laboratorio de Bioquímica y Biología Molecular, Departamento de Biología, Facultad de Ciencias, Universidad de Chile.[1].

The Glucose 6-Phosphate Dehydrogenase (G6PD) catalyzes the oxidation of glucose 6-P using NAD(P) as co-substrate. We have previously identified two amino acid residues with positive side chains (K18 and R50) as the structural determinants of the strong NADP-preference in the G6PD of Escherichia coli. In this work we generated alanine mutants of these residues to study the energetic contribution to the binding of the substrate in the transition state of the phosphate group in the cofactor, as well as the positive charges in K18 and R50. In regard to the phosphate group, the quotient between the specificity constant (k_{cat}/K_M) for NAD and NADP obtained for the K18A mutant, indicated that the phosphate group is contributing with 3.3 Kcal/mol (compared with 3.5 Kcal/mol observed for the wild-type enzyme). In these of the R50A mutant, the contribution of the phosphate moiety is lower, showing a value of 1.7 Kcal/mol. For the energetic rol of the positive side chains, the difference between the specificity constants of mutant and wild-type enzymes lead to conclude that K18 supports NADP binding with 2.1 Kcal/mol, and NAD binding with 1.8 Kcal/mol. On the other hand, the R50 side chain showed the highest contribution to NADP binding providing 2.6 Kcal/mol, but the lowest contribution to NAD binding with 0.7 Kcal/mol. Finally we built the double K18A-R50A mutant to analyze the energetic coupling of these residues in the cofactor binding, through the double cycle mutants approach. FONDECYT 1121170.

84.Molecular Characterization of Shaker Potassium Channel Inactivation. **Vergara-Jaque, A.**[1], Poblete, H.[1], Holmgren, M.[2]. Centro de Bioinformática y Simulación Molecular. Universidad de Talca.[1], National Institute of Neurological Disorders and Stroke, National Institutes of Health.USA.[2]. Sponsored by González, W.

The potassium channels encoded by the Drosophila Shaker gene are activated in response to changes in the membrane potential. After activation, the channels enter a nonconducting (or inactivated) state. Site-directed mutagenesis experiments have suggested that this inactivation is caused by the interaction of a cytoplasmic domain (N-ter) of the channel with its intracellular cavity, when the pore is open. Moreover, analyses of double-cysteine mutants have demonstrated that the A2 residue of the N-ter enters the inner vestibule in an extended configuration, to interact with the I470 residue of the channel pore blocking the ion flow. In order to characterize, at molecular level, the binding mechanism of the N-ter fragment in the Shaker K+ channel, a molecular model of this channel was constructed. Furthermore, the first 10 amino acids of the N-ter were docked into the intracellular cavity and long timescale molecular dynamics simulations were performed to determine the preferential binding sites of these amino acids with each monomer of the channel. Our results support the hypothesis that the N-ter fragment approaches the top of the inner vestibule and that its tip is positioned close to the entrance of the selectivity filter, with the amino group of the N-ter located in a potassium binding site formed by the T442 residues. Furthermore, the evaluation of the interaction of the N-ter with each monomer further revealed that this fragment can enter the channel to interact equally with any of the four monomers.

85.Phylogenetic, molecular dynamics and fluorescence studies of cofactor preference of the 6-phosphogluconate dehydrogenase from *Escherichia coli*. **Escobar, H.**[1], Maturana, P.[2], Cabrera, R.[3]. Escuela de Ingeniería en Bioinformática, Universidad de Talca[1], Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile[2], Laboratorio de Bioquímica y Biología Molecular, Departamento de Biología, Facultad de Ciencias, Universidad de Chile[3].

The 6-phosphogluconate dehydrogenase (6PGDH) is a key enzyme in the oxidative branch of the pentose phosphate pathway, involved in the oxidative decarboxylation of 6-phosphogluconate using NAD or NADP as the cofactor. 6PGDH from *E. coli* has been classified as a NADP selective enzyme, however structural studies on the binding properties and the specific role of binding side residues have not been reported. In this work we studied phylogenetic, structural and binding aspects related with the cofactor specificity of *Ec*6PGDH. First, we performed a phylogenetic tree of different 6PGDHs to determine the distribution of the cofactor specificity traits, showing clear clusters of cofactor specificity. Furthermore, we suggest that in the 6PGDH lineage, the NAD specific enzymes appear previous to the NADP specific forms that carry a C-terminal domain duplication. We studied the interactions that allow the recognition of these cofactors by molecular dynamics using the AMBER force field in explicit solvent. We performed 10 ns simulations for *Ec*6PGDH. Our analysis shows that the residues Arg34, Ser35 and Lys38 could be the main determinants of cofactor preference. Finally, the binding of NAD and NADP to 6-phoshogluconate dehydrogenase was assayed by the effect on the intrinsic fluorescence. Our work provides strong evidences for the preference of NADP in the *Ec*6PGDH.FONDECYT 1121170.

86.Structural determinants of nucleotide binding site in ribokinase of *Homo sapiens*. **Leon, M.**[1], Guixé, V.[1]. Laboratorio de Bioquímica y Biología Molecular, Departamento de Biología, Facultad de Ciencias, Universidad de Chile.[1].

Human ribokinase (RK) belongs to the ribokinase superfamily and catalyzes D-ribose phosphorylation in presence of MgATP to yield D-ribose-5-P, which is an important in the pentose-P pathway. Interestingly RK is activated by phosphate. We determined the presence a conserved threonine (T235) located at the active-site. We evaluate the role of this Threonine using site-directed mutagenesis, (T235S and T235I). Both mutants formed inclusion bodies and only T235S was found soluble with purification yields lower that the wild enzyme. Kinetic characterization of T235S showed no significant changes in kinetic parameters to be evaluated in the absence of phosphate. Surprisingly, the K_m for MgATP and ribose increase 8 and 3 times respectively in presence of phosphate, without significant changes in k_{cat}, missing phosphate activation.We also study the structural determinants for nucleotide binding by fluorescence measurements. We evaluate MgATP binding as well as GTP, CTP and UTP. In the wild type enzime K_d for MgGTP is 20 times lower than the one determined for MgATP. Finally, we investigated the interaction between the wild-type enzyme and TNP-ATP and Mant-ATP, K_d values indicate that the ribose motif of the nucleotide does not participate significantly in binding at the active site. These results indicate that T235 could be involved in the folding and phosphate activiation of human RK, the fluorescence results indicate that ribose is not directly involved in nucleotide binding to active site. (FONDECYT 1110137).

87.Modular dissection of the ribokinase super family fold. **Villalobos, P.**[1], Méndez, M.[1], Cabrera, R.[1], Babul, J.[1]. Departamento de Biología, Facultad de Ciencias, Universidad de Chile.[1].

The Ribokinase superfamily includes ATP-depend sugar kinases with a broad range of phosphoryl acceptor specificities. The superfamily fold is composed of a mayor α–β–α domain, and a minor domain that functions as a lid over the active site. The family members show structural divergences at the minor domain (insertion/deletions of complete secondary structure elements related to changes in dimeric packing and oligomeric state) and the major domain (length and packing of secondary structure elements and unstructured regions). In order to obtain a clear description of the structural diversity and to define the contribution of different regions to the folding and function of the ribokinase fold, a non-redundant set of 41 members was selected for dissection into 9 modules. A module is a contiguous segment of residues in the three-dimensional structure, representing a compact region. We quantified the contacts with donor or acceptor substrates for every module in each protein. The amino acid sequence of modules with high contact numbers (modules 1, 2 and 3) was used to construct a Bayesian phylogenetic tree. The topology of the resulting tree and phenetic dendrograms (employing Qres and Qh measurements) are in agreement to previously reported phylogenetic studies obtained by using the whole structure of non-redundant set of family members. Finally, we also analyzed the distribution of conserved Protein Core Atomic Interaction Networks (PCAIN) of residues for each module to evaluate differences with the pattern observed for the contacts with the substrate. Fondecyt 1090336.

88.Functional analyses of HCV RNA polymerase mutants in potential phosphorylation sites at the fingers subdomain, and profile of posttranslational modifications of the recombinant protein. **Hernández, S.**[1], Muñoz, E.[2], Díaz, A.[1], Loyola, A.[2], Villanueva, R.A.[1]. Departamento de Ciencias Biológicas, Universidad Andres Bello.[1], Fundacion Ciencia & Vida[2].

Hepatitis C virus (HCV) is a single-stranded RNA virus that replicates in the cytoplasm of hepatocites causing persistent infections that can progress to cirrhosis, and hepatocellular carcinoma. HCV genome encodes a RNA-dependent RNA polymerase, known as NS5B protein, which has been shown to be regulated posttranslationally by phosphorylation at its N-terminal, fingers subdomain. Using site-directed mutagenesis, we have constructed a series of mutants of HCV NS5B in which conserved serine residues at the finger subdomain were replaced either by aspartic acid or alanine in order to mimic a constitutive phosphorylation or eliminate it, respectively. The mutant NS5B proteins were expressed in bacteria with a His 6X tag at its N-terminus, and purified by affinity chromatography. The activity of the mutant enzymes were assayed on binding assays of [3H]-GTP to DE81 filters using homopolymeric templates (OligoG/PolyC) or viral templates. Aditionally, we have generated recombinant baculoviruses encoding the HCV NS5B, which was purified from baculovirus-infected insect cells. Utilizing mass spectrometry of the purified protein, a full profile of posttranslational modifications of the HCV polymerase was obtained. Together, our results will help to understand the regulation of HCV RNA polymerase by posttranslational modifications.Funded by FONDECYT 1100200, and MECESUP UAB0802.

89.Identification, sequencing, heterologous expression and characterization of a α-D-galactopyranosidase from the pectinolytic fungus *Penicillium purpurogenum*. **Faúndez, C.**[1,2], Eyzaguirre, J.[1]. Universidad Andrés Bello[1], Universidad de Santiago de Chile[2].

The filamentous fungus *Penicillium purpurogenum* has demonstrated its ability to degrade plant cell walls by means of cellulolytic, xylanolytic and pectinolytic activities. Among the reported enzymes that allow pectin degradation, the α-D-galactopyranosidase activity has not been described in this fungus. Specifically, this enzyme may act on the highly substituted pectin regions. The main purposes of this study are the identification, sequencing, heterologous expression and characterization of a α-D-galactopyranosidase from *P. purpurogenum*. For this purpose, the fungus was grown on sugar beet pulp (which has a high pectin content) as a carbon source and the α-D-galactopyranosidase activity was determined. A partial purification of the enzyme was performed by chromatography on Cibacron Blue-Sepharose at pH 4.0 and a DEAE- Sephadex A-50 at pH 7.0. Proteins were visualized by SDS-PAGE and the bands obtained from the last column were analyzed by mass spectrometry. The peptides obtained were searched in the database of the *P. purpurogenum* draft genome. We identified a gene that may encode a putative α-D-galactopyranosidase. This gen has 1650bp and the cDNA has 1296bp. The cDNA was expressed in *Pichia pastoris*, and the enzyme was subjected to characterization. The enzyme belongs to glycosyl hydrolase family 27 with a theoretical molecular mass of 45.7 kDa and a theoretical isoelectric point of 5.15. The optimum pH for activity ranges between 3.0 and 3.5, and the optimum temperature is about 45°C. Grant support: FONDECYT N° 1100084, UNAB DI-61-12/R.

90.Hydrogen peroxide affects the activity and thermal stability of Glutamyl-tRNA Reductase from *Acidithiobacillus ferrooxidans*. **Farah, C.**[1], Ibba, M.[2], Orellana, O.[1]. Programa de Biología Celular y Molecular, Facultad de Medicina, Universidad de Chile [1], Microbiology Department, The Ohio State University [2].

The initial reaction of heme biosynthesis in gamma proteobacteria is catalyzed by the glutamyl-tRNA reductase (GluTR). This enzyme reduces the carboxyl group of glutamate moiety from Glutamyl-tRNA using NADPH to form glutamate semialdehyde (GSA). GSA is then converted to aminolevulinic acid (ALA), the universal precursor of the biosynthesis of heme, by the glutamate semialdehyde amidotransferase (GSAM). Historically the investigation on GluTR has been difficult because the tendency of the enzyme to aggregate and precipitate when it is overexpressed in *Escherichia coli* from the corresponding gene from various species. Here, we describe the effect of hydrogen peroxide on the activity of GluTR from *Acidithiobacillus ferrooxidans*, a soluble dimeric enzyme that binds heme. We found that hydrogen peroxide inactivates GluTR and this correlates with the decrease in the thermal stability and the oxidation of cysteine residues of the enzyme. Interestingly, binding of heme to GluTR does not affect the inactivation rate of GluTR by hydrogen peroxide, suggesting that heme is not involved in the oxidation of this enzyme, as occurs in other proteins. We hypothesize that the inactivation of GluTR by hydrogen peroxide correlates with the decrease in the heme content in *A. ferrooxidans* exposed to hydrogen peroxide. Supported by Fondecyt 1110203 (OO) and Mecesup UCH0714 (CF).

91.Tellurite-mediated oxidative stress damages the activity of *Escherichia coli* terminal oxidases under aerobic conditions. **Abarca Lagunas, M.J.**[1], Díaz Vásquez, W.A.[1], Cornejo Leiva, F.A.[1], Vásquez Guzmán, C.C.[1]. Universidad de Santiago de Chile[1].

Tellurite is toxic to most bacteria and its effects are associated in part with the generation of reactive oxygen species (ROS) that in turn attack multiple cell targets, including the glycolytic pathway, enzymes from the Krebs cycle, etc. However, very little is known to date about tellurite effects on the electron transport chain (ETC). Have been observed that tellurite decreases ATP levels and α pH in the ETC suggesting that it probably represents another target of the tellurium oxyanion.In this work, the particular effect of tellurite on the *E. coli bo* and *bd-I* cytochrome oxidase complexes was studied under aerobic conditions. It was found that cell exposure to sublethal tellurite concentrations results in the establishment of an oxidative stress status that affects the normal ETC functioning, with decreased activity of the terminal oxidases, particularly the cytochome *bo* complex. Decreased expression of genes encoding this complex was also observed, showing that tellurite affects its activity and also their transcripts level. As expected, induction of genes involved in the response to oxidative stress was observed. However and given the great toxicity of tellurite, the cell changes drastically to an anaerobic-like metabolism to alleviate the effects derived from tellurite exposure. Finally, it was determined that *E. coli* membranes are able to reduce tellurite in a NADH-dependent, KCN-insensitive reaction, suggesting that terminal oxidases are not involved.Funding: FONDECYT 1090097, Scholarships Doctoral Thesis Support 24121087, DICYT and VRID USACH.

92.Evaluation in silico of the copper-binding ability of a Kunitz trypsin inhibitor in poplars (*Populus* spp). **Valenzuela Riffo, F.**[1], Guerra, F.[2]. Escuela de Ingeniería en Bioinformática, Universidad de Talca[1], Instituto de Biología Vegetal y Biotecnología, Universidad de Talca[2]. Sponsored by Martínez Oyanedel, J.

Copper is an essential micronutrient for plants, but in excess it can cause negative effects. The tolerance to this metal is important for developing suitable plants for phytoremediation systems. Recently, studies assessing poplars (Populus spp) under copper stress have identified genes significantly up-regulated in response to excess of this metal. Some of them encode Kunitz trypsin inhibitors (PdKTI3). This study aimed to evaluate in silico the role of PdKTI3 as a possible element of tolerance to copper stress, by its ability of chellating free metal ions. The evaluation included the analysis of the copper-binding ability of PdKTI3 protein, through the modeling by homology of tridimensional structure (3D). KTI3 ESTs were aligned against existing sequences of the KTI3 family in poplar, available in databases. Gene full-length sequences were used to predict the 3D structure of the protein and assessing its ability to interact with copper, by using different bioinformatics software. The results indicated the ability of PdKTI3 to bind copper. Additional studies are being conducted to confirm in vivo the effect of the overexpression of the gene PdKTI3 in plants.

93.Characterisation of VvSDL and VvS6PDL, enzymes potentially involved in sorbitol metabolism in grapevine (*Vitis vinifera*). Araya, J.[1], Handford, M.[1]. Laboratorio de Biología Molecular Vegetal, Facultad de Ciencias, Universidad de Chile[1].
Sorbitol is the main photosynthetic product and form in which carbon is phloem-translocated in the Rosaceae family, which includes peaches, pears and apples. Glucose is metabolised to sorbitol via NADPH-dependent sorbitol-6-phosphate dehydrogenase (S6PDH) and once in sink organs, a proportion of this sugar alcohol is metabolised to fructose via NAD-dependent sorbitol dehydrogenase (SDH). Nevertheless, SDH has also been found in non-Rosaceae species, such as tomato, maize and *Arabidopsis*, in which the main phloem-translocated carbon is sucrose. Sorbitol presence in non-Rosaceae species has been linked to stress conditions, such as drought or stress due to high or low temperatures. As a first approach to determine the physiological role of sorbitol and SDH in these species, we have identified a putative SDH and S6PDH from grapevine (*Vitis vinifera*) which we named VvSDL and VvS6PDL respectively. Both possess all the molecular features and the conserved domains of the characterised enzymes. In silico analysis and qRT-PCR reveals that *VvSDL* is expressed differentially during the development of grape berries. In order to determine its enzymatic activity, VvSDL was cloned, and heterologously expressed and purified from *E. coli*. In order to determine the subcellular localisation tobacco leaves were transiently transformed using *Agrobacterium* harboring GFP-VvSDL. The results show that VvSDL is a cytosolic protein which is able to oxidise sorbitol in the presence of NAD+ in vitro. Additionally, *VvS6PDL* is expressed in all development states of grape berries studied. Funding: Innova 07CN13PBD-19.

94.Molecular modeling of NAC42 transcription factor and the binding to specific cis-regulatory elements. Soto, F.[1], Caballero, J.[1], González, W.[1]. Universidad de Talca[1].
Transcription factors are key players in the regulation of gene expression and, hence, are essential for cell function. In this work we quantitatively assess the binding affinity of ANAC042 transcription factor to different cis-regulatory elements of DNA by docking simulations. NAC proteins represent one of the largest families of transcription factors in land plants and participate in embryonic development process, root growth, signaling, stress response and senescence. NAC is an acronym of three transcription factors of this family: NAM (no apical meristem), ATAF1, 2 and CUC2 (cup-shaped cotyledon). Proteins of NAC family form functional dimers to bind DNA through one side of the structure which is positively charged. We calculated the binding affinity of ANAC042 transcription factor to DNA in silico through a protein-DNA docking. The binding energies indicate which parts of the NAC transcription factor binds DNA in vivo. To achieve this task, we generated homology models of ANAC042 transcription factor based on the crystal structure of ANAC019, calculated the electrostatic potential and thus corroborate the location of the face in the dimer that interacts with DNA. Later, we generated 3D models of the DNA cis-regulatory elements, and carried out the docking between these elements and NAC TFs to determine the binding energy between them. We corroborated that the zone ANAC042 that could bind the DNA are the residues Leu117 to Val168 (monomer A) and Ser288 to Val336 (monomer B) placed in the NAC domain of the protein.

95.Sequencing, expression in *Pichia pastoris* GS115 and characterization of a pectin lyase from *Penicillium purpurogenum*. Pérez, C.[1], Eyzaguirre, J.[1]. Universidad Andrés Bello[1].
Microorganisms, due to their secretory capacity, have great potential. They produce enzymes which have been exploited commercially in the food industries, such as in quality wine production and clarification of fruit juices. Many of them are pectinases, a heterogeneous group of related enzymes that degrade pectin, a plant cell component. Our laboratory has been concerned with the pectinolytic system from *Penicillium purpurogenum*. This fungus, when grown on sugar beet pulp, secretes to the culture medium pectin lyase (EC. 4.2.2.10). This enzyme is able to act on highly esterified pectins and does not require Ca?2+ for activation. It catalyzes the cleavage of ?-1,4-glycosidic bonds between methoxylated residues of D-galacturonic acid by means of ?-elimination and formation of ?-4,5-unsaturated products. For this purpose the gene, cDNA and the promoter region of a putative pectin lyase were sequenced, based on a peptide obtained by mass spectrometry. Three introns of 61, 59 and 83pb respectively, were detected. The cDNA was heterologously expressed in *Pichia pastoris* selecting clones with zeocin resistance. Pectin lyase was purified from culture supernatants using hydrophobic interaction chromatography and characterized. Optimum pH and temperature for the enzyme are 6.0 and 50°C, respectively. The enzyme has a molecular mass of 42 KDa by SDS polyacrylamide gel electrophoresis. Its 3D structure has been predicted by I-TASSER, and is similar to a pectin lyase from *Aspergillus niger*. This work has been supported by grants from FONDECYT (1100084) and UNAB (DI-61-12/R).

96.The deletion of 38 amino acids of the C-terminal and the sited-directed mutagenesis of Ala19Tyr decrease the Feroxidase activity but improve the formation of a homogeneous iron core in *Cholorobium tepidum* Ferritin. Yévenes, A.[1], Brito, C.[2], Márquez, V.[2], Sandoval, C.[2], González-Nilo, F.[2], López-Castro, J.[3], Domínguez-Vera, J.M.[4], Watt, R.[5]. Pontificia Universidad Católica de Chile[1], Universidad Andrés Bello[2], Universidad de Cadiz[3], Universidad de Granada[4], Brigham Young University[5].
Ferritins are large protein cages (10-12 nm diameters) that control the reversible formation of iron-oxy biominerals in a cavity with a diameter of 6-8 nm. The cavity communicates with the solvent through six hydrophobic channels along the 4-fold symmetry axis, and eight hydrophilic channels along the 3-fold axis. *Chlorobium tepidum* Ferritin is able to store iron, but the iron storage capacity and ferroxidase activity is less than that reported for Ferritins from other organisms. The *C. tepidum* Ferritin has an unusual C-terminal region and our homology model predicts that this region projects inside the protein cage. In order to evaluate the role of the long C-terminal region and the role of the conserved Tyr19 in the activity of Ferritin we introduced by site-directed mutagenesis a stop codon in position 166 and a Tyr residue instead Ala. The intrinsic fluorescence and the transmition electron microscopy analysis shows that the modifications introduced in the *C. tepidum* Ferritin did not affect the tertiary and the quaternary structure of the protein. However, the ferroxidase activity of the mutated proteins decreased as a consequence of the modifications introduced. Our results show that the long C-terminal region is important to the function of the ferroxidase center, however, the analysis by High Angle Annular Dark Field microscopy shows that the absence of the C-terminal region improves the formation of a homogeneous iron core in *C. tepidum* Ferritin. Supported by Facultad de Química, Pontificia Universidad Católica de Chile.

97.Shotgun proteomics analysis of *Penicillium purpurogenum* secretome; identification of lignocellulolytic enzymes. **Mardones, W.**[1], Eyzaguirre, J.[1], Callegari, E.[2]. Universidad Andrés Bello[1], Universidad de Dakota del Sur, Estados Unidos[2].
Lignocellulosic biomass (component of plant cell walls) is a renewable energy source, and the enzymes that are involved in its degradation are interesting for possible biotechnological applications. Numerous filamentous fungi produce a variety of lignocellulolytic enzymes and are capable of regulating their expression depending on the composition of the carbon source, thus allowing for an efficient degradation of the lignocellulosic biomass. Our goal is to identify the enzymes secreted by the lignocellulolytic fungus *P. purpurogenum* when grown on sugar beet pulp (rich in cellulose and pectin) as carbon source. For this purpose we utilized bidimensional nano chromatography coupled to mass spectrometry in tandem (2D nano-LC MS/MS). This is a "gel free" proteomics technique that allows the identification of proteins in a complex sample. Utilizing 2D nano-LC MS/MS and an "in-house" protein database (derived from a draft of the genome of *P. purpurogenum*), we have identified 52 different secreted proteins, of which 42 are probably related to the degradation of lignocellulose. We were able identify putative cellulases, pectinases, arabinanases, peptidases, beta-galactosidases, and others. All activities indicated above are necessary for the degradation of cellulose and pectin. This work identifies novel enzymes which may be useful in biotechnological processes, and contributes to a better understanding of lignocellulose degradation. Grant support: FONDECYT 1100084; UNAB DI-31-12/I and DI-61-12/R; MECESUP UAB0802.

98.Molecular dynamics simulations of the binding of phosphorylcholine (Pch) to phosphorylcholine phosphatase (PchP). **Bustos, D.**[1], Vergara-Jaque, A.[1], Beassoni, P.[2], Alzate-Morales, J.[1]. Centro de Bioinformática y Simulación Molecular. Universidad de Talca.[1], Departamento de Biología Molecular, Universidad Nacional Rio Cuarto, Córdoba, Argentina.[2]. Sponsored by Gónzalez, W.
Pseudomonas aeruginosa is an opportunistic pathogen with high antibiotic resistance, involved in serious complications of the respiratory tract in patients with cystic fibrosis or burns. *Phosphorylcholine phosphatase* (PchP) has been implicated in the pathogenesis of this bacterium through its sequential and coordinated action with the enzyme *hemolytic phospholipase C* (PlcH), hydrolyzing phosphorylcholine (Pch) to produce choline and phosphate, nutrients that support bacterial growth. PchP belongs to the HAD superfamily because it contains conserved motifs, which fold to form the catalytic site that binds Mg+2 and the phosphate moiety of the Pch sustrate; however, the residues that interact with the alkylammonium region are still unknown. Comparisons with choline-binding proteins and site-directed mutagenesis have demonstrated that the residues 42E, 43E and the aromatic triplet 82YYY84 could be implicated. Changes of these residues affect substrate affinity and the inhibitory effect produced by high Pch concentrations, allowing us to postulate that two sites, one catalytic and one inhibitory, are present In PchP and share the residues mentioned above. On the basis of this evidence, we built a molecular model of PchP and employed molecular dynamics simulations to analyze the binding of Pch to the protein and to characterize the binding sites resulting from the folding of the amino acid motifs. The simulations further reveal the interactions that govern the affinity of the Pch in both binding sites and provide key information for the design of inhibitors that minimize the pathogenic effect of the bacterium.

99.*In vitro* Biochemical and kinetic characterization and *in silico* studies of Ferredoxin NADP+ Reductase from *Gracilaria chilensis*. **Vorphal, M.**[1], Martínez, J.[1], Bunster, M.[1]. Laboratorio de Biofísica Molecular, Departamento de Bioquimica y Biologia Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción[1].
Ferredoxin NADP+ oxidoreductase (FNR) is a photosynthetic flavoenzyme that catalyzes the electron transfer between ferredoxin and NADPH. In higher plants, this enzyme is associated to the stromal side of the thylakoid membrane in the chloroplast; in red algae and cyanobacteria, this enzyme is structurally associated with phycobilisomes (PBS), which are light-harvesting complexes in these organisms, some of them which present isoforms of the enzyme that are associated to PBS, and the interaction is dependent of FNR N-terminal domain that localize the enzyme in the phycobllisomes. In *Gracilaria chilensis*, a red algae, FNR was obtained from soluble extract and from purified phycobilisomes, using differential ultracentrifugation and density gradient centrifugation. Western blot analyses and zymogram on the native-PAGE and SDS-PAGE of the samples were used for the characterization of the enzyme. For the kinetic characterization, the oxidation of NADPH to NADP+ was followed at 340nm to obtain the kinetic parameters. The FNR from *Gracilaria chilensis* was found to have a molecular weight of 34kDa. A molecular model, not reported until now for this enzyme in Gracilaria sp, was built based on a bioinformatic study of homologous sequences and structures. In this work, a structural comparative analysis between FNR from different species is also reported. Proyecto DIUC 211.037.012-1.0.

100.Heterologous expression of a *Penicillium purpurogenum* beta-D-xylosidase in *Pichia pastoris* and its characterization. **Ravanal, M.C.**[1], Eyzaguirre, J.[1]. Universidad Andrés Bello[1].
Lignocellulose is the major component of plant cell walls and it represents a great source of renewable organic matter. Lignocellulose is composed of lignin, pectin, cellulose and hemicelluloses. The fungus *Penicillium purpurogenum* when grown under any of several carbon sources secretes to the culture medium beta-D-xylosidases (E.C 3.2.1.37). This work focuses on the heterologous expression of a beta-D-xylosidase from *Penicillium purpurogenum* in *Pichia pastoris* GS115. For this purpose, plasmid pPICZB, containing the beta-D-xylosidase gene (including a polyhistidine tag) was used. The beta-D-xylosidase gene and its cDNA were sequenced; no introns were found, and their size is 1011 pb. The recombinant enzyme was purified to homogeneity with a Ni-NTA column and characterized; the molecular weight determined by SDS-PAGE is approximately 49,000 and the isoelectric point (pI) obtained by isoelectrofocusing is about 5.1. When assayed with p-nitrophenyl beta-D-xylopiranoside (pNPXyl) the enzyme followed Michaelis-Menten kinetics with a Km of 0.55. The optimum pH is 6.0 and the optimal temperature is 40 °C. The beta-D-xylosidase acts on short xylooligoshacharides (X2-X5) and when assayed for the liberation of xylose from natural substrates such as arabinoxylan a synergism between beta-D-xylosidase and endoxylanase A from *P. purpurogenum* was observed. This effect was detected by TLC and quantified enzymatically. Based on sequence similarities the newly described beta-D-xylosidase can be classified in family 43 of the glycosyl hydrolases. This work has been supported by grants from FONDECYT (1100084), UNAB (DI-61-12/R) and FONDECYT Post-Doctoral Fellowship (3120032).

101.C/EBPbeta-LAP*-mediated regulation of osteoblast lineage gene transcription: functional interaction with the SWI/SNF chromatin remodeling complex. **Meza, D.**[1], Aguilar, R.[1], Sepulveda, H.[1], Montecino, M.[1]. Center for Biomedical Research and FONDAP Center for Genome Regulation, Faculty of Biological Sciences and Faculty of Medicine, Universidad Andres Bello, Santiago, Chile[1].
C/EBPbeta transcription factor is essential during control of cellular proliferation and differentiation. This factor is expressed as three isoforms (LAP*, LAP and LIP) that are generated by alternative translation initiation and differing in its N-terminus. During osteoblast differentiation, the relative abundance between CEBPbeta isoforms changes in a stage-dependent manner: LAP is the main isoform in proliferating pre-osteoblasts whereas LAP* expression appears as the differentiation proceeds. Recently, our group demonstrated that C/EBPbeta-LAP* down-regulates Ric-8B gene expression in osteoblasts. This inhibition involves recruitment of the SWI/SNF chromatin remodeling complex to the Ric-8B promoter region in a C/EBPbeta-LAP*-dependent manner. In addition, it has been shown that interaction between C/EBPbeta-LAP* and SWI/SNF is impaired by di-methylation of the arginine 3 residue of LAP*. Using co-immunoprecipitation we find that the mutation R3L at C/EBPbeta-LAP*, which mimics a di-methylation at this residue, impairs its ability to interact with SWI/SNF in osteoblastic cells. To now assess whole genome targets that are globally recognized by C/EBPbeta-LAP* during osteoblast differentiation and the dependency on the co-recruitment of SWI/SNF, we have generated stable cell lines derived from rat osteosarcoma ROS17/2.8 cells which express C/EBPbeta-LAP* or C/EBPbeta-LAP*R3L under control of tet-off system. Using this valuable tool we confirmed specific gene targets recognized by this factor which result either activated or repressed in a SWI-SNF-dependent manner. FONDECYT1095075 and FONDAP15090007.

102.Sequencing and characterization of a recombinant endopolygalacturonase of *Aspergillus fumigatus* heterologously expressed in *Pichia pastoris* GS115. **Pérez, N.**[1], Eyzaguirre, J.[1]. Universidad Andrés Bello[1].
The enzymatic degradation of pectin is a complex process involving many enzyme activities produced mainly by bacteria and fungi. Hydrolysis of the main chain of the pectin in the "smooth" region, the homogalacturonan, is carried out mainly by polygalacturonases. These enzymes can be classified as members of the family 28 of the glycosyl hydrolases. The pectinolytic system of the fungus *Aspergillus fumigatus* is poorly understood; therefore the enzymatic strategies used by the fungus in order to survive when facing different environments are interesting to study. An endopolygalacturonase of *A. fumigatus* was sequenced, heterologously expressed in *P. pastoris* and characterized. The gene is 1238 bp long with two introns, and codes for a protein of 368 amino acid residues with a theoretical pI and molecular mass of 4.1 and 36.2 kDa respectively. SDS-PAGE and sugar content analysis show that the recombinant enzyme has a molecular mass of 52 kDa with a high sugar content of about 80%. The optimal pH is 4 and the optimal temperature is between 40 to 50?C. Polygalacturonic acid appears to be the preferred substrate of the enzyme in contrast to trigalacturonic acid, esterified or deesterified pectin. When testing the thermal stability, the enzyme retains 50% of the remaining activity after incubation for 1 hour at 60?C. The acid character and high thermal stability makes this enzyme a good candidate for applications in bio-industrial processes. Grant support: FONDECYT Nº 1100084, UNAB DI-61-12/R.

103.Circadian rhythms in Botrytis cinerea: exploring the effect of the FRQ clock protein in the pathogenicity of Botrytis cinerea. **Canessa, P.**[1], Hevia, M.[1], Larrondo, L.[1]. Depto. Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.[1].
It has been recently suggested that the circadian clock may modulate the outcome of the plant-pathogen interaction. While this cellular timekeeper is known to be present in plants, modulating jasmonate-mediated defense in Arabidopsis, little is known about the molecular mechanisms behind the aforementioned interaction in necrotrophic fungi. Moreover, besides the studies in *Neurospora crassa*, there has been no data proving the existence of circadian clocks in fungi other than Neurospora. *Botrytis cinerea* is a necrotrophic fungus that infects over 200 plant species, and we have hypothesized the existence of a functional circadian clock. Thus, using comparative genomics based on the fungal circadian model *N. crassa* we have started the characterization of the circadian oscillator of *B. cinerea*. In Neurospora, the FRQ protein is central for the circadian clock, being the master oscillatory protein whose gene expression is tightly controlled by the White-Collar complex. Using a translational luciferase reporter, we have observed oscillatory levels of the BcFRQ protein, while RT-qPCR experiments have confirmed these daily oscillations in *Bcfrq* expression under constant dark conditions. Genetic data indicates that both BcFRQ and *Bcfrq* respond to light pulses, while under oscillatory culture conditions, BcFRQ anticipates cyclical-environmental changes, a key characteristic of circadian behavior. In addition, we have generated a delta-*Bcfrq* strain using homologous recombination. Pathogenicity tests performed using Arabidopsis and *Phaseolus vulgaris* indicate the participation of the FRQ protein in the pathogenicity process. Fondecyt-postdoc 3110127 to PC and Fondecyt 1090513, ICGEB-CRP CHI09-02 to LFL.

104.Docking and biological studies of coumarins derivatives as lipoxygenase inhibitors. **Silva, F.**[1], Núñez, C.[1], Mascayano, C.[1], García-Beltrán, O.[2], Cassels, B.[2], Fierro, A.[3]. Departamento de Ciencias del Ambiente, Facultad de Química y Biología, Universidad de Santiago de Chile[1], Departamento de Química, Facultad de Ciencias, Universidad de Chile, Santiago, Chile[2], Departamento de Química Orgánica, Facultad de Química, Universidad Católica de Chile, Santiago, Chile[3]. Sponsored by González -Nilo, D.
The biological properties of lipoxygenases (LOX) have been widely studied because they are involved in the biosynthesis of Leukotrienes (LTs) and Lipoxins (LPs). Several pathologies including: prostatic cancer and asthma (5-hLOX), breast cancer and psoriasis (12-hLOX) and colorectal cancer and atheriosclerosis (15-hLOX) have been related with yours isoforms. Coumarins, benzopyrone derivatives, display activities as antioxidant, anticancer, antiviral and anti-inflammatory activity and contain some structural requirements that are important to be used as LOX inhibitors.In this research a systematic study of coumarins derivatives using molecular simulation and biological assays was done in order to understand the binding modes and to obtain mechanistic insights. AcknowledgmentsFinancial support from Fondecyt #1120379 and Fondecyt #11085002 is gratefully acknowledged.

105.Development of a multigene classifier for the diagnosis indeterminate thyroid nodules. **Martínez S., R.**[1], Véliz G., L.[1], Urra G., S.[1], Vargas S., S.[1], Fischer O., M.[1], Kalergis M., A.[2,3,4], González D., H.[1,5]. Departamento de Cirugía Oncológica, Facultad de Medicina, Pontificia Universidad Católica, Santiago, Chile.[1], Millennium Institute on Immunology and Immunotherapy, Santiago, Chile.[2], Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontifica Universidad Católica, Santiago, Chile.[3], Departamento de Reumatología, Facultad de Medicina, Pontificia Universidad Católica, Santiago, Chile[4], Millennium Institute on Immunology and Immunotherapy[5].
Fine needle aspirate (FNA) is used routinely for clinical evaluation of thyroid nodules. However, 20% of FNA biopsies are reported to be indeterminate, thus can be either, benign or malignant. Usually, these patients undergo surgery due to a 20 % risk of cancer, which means that 80% follow an unnecessary removal of the thyroid. Thus, additional testing methods are urgently required to properly classify these patients and avoid surgery. To develop a multigene classifier that could improve the diagnosis of indeterminate thyroid nodules, an extensive search for biomarkers related to thyroid cancer was performed in PUBMED. Twenty genes were pre-selected as candidates. Gene expression was determined by Real Time PCR (qPCR) and the cT values were used to generate an algorithm using the STAR Bioinformatic software. The resultant algorithm was used to generate an integrated receiver operative curve (ROC) for the selected gene arrangement. As a training set of the algorithm, mRNA was obtained from 46 papillary thyroid cancer biopsies and 36 benign nodules. Individually, our selected genes generated ROC curves with areas under the curve (AUC) ranging between 0.65-0.88. Our training set generated over 80 million gene combinations. New algorithms resulted in ROC curves with AUC between 0.88-0.93. Greatest specificity with maximal sensitivity was the criteria to choose our optimal algorithm, which presented 96% sensitivity and 93% specificity (AUC 0.93). We have generated a multigene classifier that could improve the indeterminate nodules diagnosis.

107.Inhibition of HIV-1 cap-independent translation initiation by eIF5A hypusination inhibitors. **Caceres, C.J.**[1], Pino, K.[1], López-Lastra, M.[1]. Laboratorio de Virología Molecular, Instituto Milenio de inmunología e Inmunoterapia, Centro de investigaciones medicas, Escuela de Medicina, Pontificia Universidad Católica De Chile[1].
The 5`-untranslated region (5`UTR) of the human immunodeficiency virus type 1 (HIV-1) harbors an internal ribosome entry site (IRES) that drives synthesis of the Gag polyprotein. The mRNA of HIV-1 requires cellular factors for specific tasks within the viral lifecycle such as RNA transport and gene expression. One such factors is the eukaryotic initiation factor 5A (eIF5A), a protein associated with cellular RNA transport, gene expression and cell viability. EIF5A is the only known protein to contain the hypusine, aminoacid essential for eIF5A function. In order to better understand the relationship between hipusinated eIF5A and HIV-1 IRES activity, the compounds Ciclopirox (CAS: 41621-49-2) and Deferiprone (CAS: 30652-11-0) were used to inactivate eIF5A by preventing its hypusination. Using these drugs we would establish that eIF5A modulates the HIV-1 IRES mediated translation initiation since in absence of hipusinated eIF5A the translation is considerably reduced. Results showed that the reduction in gene expression is not a consequence of a citotoxic or transcriptional effect. Using bicistronic vectors we further characterized the effect of eIF5A on IRES activity in cells. In these experiments the inhibition of IRES mediated translation initiation by Ciclopirox of Deferiprone showed to be dose-dependent. While IRES activity is hindered by the drugs, results also show that the cap dependent translation is not affected suggesting a specific relationship between eIF5A hypusination and the activity of the HIV-1 IRES. FONDECYT 1090318 y P09/016-F Iniciativa Científica Milenio del Ministerio de Economía, Fomento y Turismo.

106.The differential expression profile of CXCR3 receptor spliced variants could contribute to malignant phenotype observed in Papillary Thyroid Cancer. **Fischer , M.**[1], Urra , S.[1], Martínez., R.[1], Catalán, T.[2], Vargas, S.[1], Véliz. , L.[1], Kalergis, A.[3,4,5], Riedel, C.[6], Gónzalez, H.[1]. Departamento de Cirugía Oncológica, Facultad de Medicina, Pontificia Universidad Católica, Santiago, Chile[1], Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontifica Universidad Católica, Santiago, Chile[2], Millennium Institute on Immunology and Immunotherapy, Santiago, Chile.[3], Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontifica Universidad Católica, Santiago, Chile.[4], Departamento de Reumatología, Facultad de Medicina, Pontificia Universidad Católica, Santiago, Chile[5], Facultad de Ciencias Biológicas, Universidad Andrés Bello, Santiago, Chile[6].
Papillary thyroid carcinoma (PTC) is the most common malignancy of the thyroid gland, and frequently displays high lymph node metastases. Various chemokines are up-regulated in cancer tumors suggesting an important role in tumor progression. CXCR3-binding chemokines promote diverse cellular responses. This diversity is explained, in part, by the presence of two receptor splice variants. CXCR3A promotes growth and cellular migration whereas CXCR3B induces apoptosis. In fact, CXCR3A is identified as the pro-tumoral isoform meanwhile CXCR3B as the "suppressor tumor" variant. We have previously shown, by quantitative immunohistochemical analysis, that CXCR3 is up-regulated in human (PTC) biopsies. In order to analyze if the thyroid cancer phenotype could be represented by an unbalanced expression levels of CXCR3 variants, we examined by Real Time PCR, CXCR3A and CXCR3B mRNA expression in 50 PTC biopsies and in 15 non-malignant thyroid nodules.We found significant decreased mRNA levels for CXCR3 variants in PTC tumor samples when they were matched to non-malignant tissue from the contralateral thyroid lobe. However, when they were compared to benign nodule, CXCR3A levels were increased by 3 fold while CXCR3-B remained unchanged. Further casting of these variants in human thyroid cell lines revealed CXCR3B expression in normal epithelial thyroid cells as well as in TPC1 (thyroid cancer line) meanwhile CXCR3A transcripts were only detectable in TPC1.The differential mRNA expression profile of CXCR3 variants reported here in PTC patients could promote tumor progression mediated by antagonistic signaling transduction of CXCR3 variants.

108.Evolutionary history of the *SPARC* gene: ancestral functions and recruitment into the vertebrate skeletal cell genetic program. **Hanna, P.**[1], Bertin, A.[1], Aldea, D.[1], Otárola, G.[1], Muñoz, D.[1], Sachs, L.[2], Buisine, N.[2], Hitoyoshi, Y.[3], Hudson, C.[3], Fuentealba, J.[1], Torrejón, M.[1], Escrivá, H.[3], Bertrand, S.[3], Marcellini, S.[1]. Faculty of Biological Sciences, University of Concepción, Chile[1], Muséum National d'Histoire Naturelle, CNRS, France[2], Université Pierre et Marie Curie, CNRS, France[3]. Sponsored by Gutiérrez, L.
The *SPARC* gene codes for an extracellular protein expressed in several cell types including skeletal cells such as osteoblasts and osteocytes. The SPARC protein binds calcium ions and acts as a collagen chaperone, thereby contributing to the biomineralization of bone extracellular matrix. Here, we sought to 1) shed light on the expression of *SPARC* in chordate embryos prior to its recruitment in skeletal cells and 2) determine its expression pattern and transcriptional regulation in vertebrate osteoblasts. 1) Whole mount in situ hybridizations performed on embryos from non-vertebrate species (tunicates and amphioxus) as well as vertebrates (*Xenopus tropicalis*) revealed that the ancestral expression pattern of *SPARC* was predominantly mesodermal in domains that include the developing somites and the notochord. 2) In order to shed light on the transcriptional changes that led to the recruitment of *SPARC* into the vertebrate skeletal cell genetic program, we first performed RT-PCR, in situ hybridizations and RNA-seq on *Xenopus tropicalis* endochondral as well as intramembraneous bones. Our results demonstrate an expression at all steps of the osteogenic lineage, from undifferentiated mesenchyme to osteoblasts and osteocytes. Because genomic comparisons between human and amphibian sequences did not detect significant conservation outside coding exons, we mapped the *SPARC* promoter by RNA-pet and characterized its epigenetic signatures by ChIP-qPCR. This work represents a first step toward the understanding of how the regulatory landscaped of *SPARC* was shaped to allow its expression in skeletal cells during vertebrate evolution. Funding: FONDECYT 1110756.

109.The distance between a nucleosome and a transcription factor binding site influences targeted nucleosome eviction activity catalyzed by the SWI/SNF complex. **Alarcón, V.**[1], Hepp, M.I.[1], Gutiérrez, J.L.[1]. Laboratorio de Regulación Transcripcional, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile.[1].

Binding of transcription factors and RNA polymerase to gene promoters determines transcriptional activity. Changes of DNA sequences in gene promoters leading to alterations in binding of transcription factors, distribution of regulatory sequences or DNA methylation status are factors that could affect transcriptional activation. In this context, the accessibility of regulatory proteins can be modulated by the distribution of nucleosomes at gene regulatory regions. The chromatin structure state can be altered by ATP-dependent chromatin remodeling complexes (e.g. SWI/SNF, RSC) and several transcription factors can target these complexes to gene regulatory regions. It is unknown whether a variation in the distance between a bound transcription factor and a nucleosome would influence the targeted nucleosome remodeling activity of these ATP-dependent machines. In order to ascertain this question we performed *in vitro* nucleosome remodeling assays using different mononucleosome probes, varying in the distance between a transcription factor-binding site (Gal4) and a positioned nucleosome, the yeast SWI/SNF complex and the chimeric transcription factor Gal4-VP16. Under our assay conditions SWI/SNF had an absolute requirement of Gal4-VP16 for reaching the mononucleosome probe. Our results shown that nucleosome disassembly was higher when shorter distances between the Gal4 binding site and the positioned nucleosome were present. We also found that the extension of extranucleosomal DNA influences the strength of SWI/SNF binding to the nucleosomes. Our results point to a new aspect that could have an impact on gene expression at the level of transcriptional regulation. Fondecyt-1085092; DIUC-211.037.014-1.0.

110.Insulin-increased human equilibrative nucleoside transporter 2 involves Srebp-1c increased expression in human umbilical vein endothelial cells. **Pardo, F.**[1], Westermeier, F.[1], Salomon , C.[1], Guzmán-Gutierrez , E.[1], Arroyo, P.[1], Santos, M.[1], Leiva, A.[1], Sobrevía, L.[1]. Cellular and Molecular Physiology Laboratory (CMPL), Division of Obstetrics and Gynecology, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile. [1].

Human umbilical vein endothelial cells (HUVEC) take up adenosine via human equilibrative nucleoside transporters 1 (hENT1) and hENT2. Insulin increases hENT2 expression and activity in HUVEC via unknown mechanisms. Since sterol regulatory element-binding protein 1c (Srebp-1c) mediates insulin effect in other cell types, we studied whether insulin effect on *SLC29A2* (for hENT2) promoter activity involves this transcription factor in HUVEC. Fragments of *SLC29A2* promoter region were generated (pGL3-hENT2 constructs, -1998, -1491, -1086, -864 and -602 bp from ATG) including an -1129C<A mutation (pGL3-hENT2-1491mut) construct for Srebp binding site. HUVEC were electroporated (320 V, 975 microF, 9-11 ms) and then exposed to insulin (1 nM, 8 hours) and luciferase reporter activity was measured. Srebp-1c protein abundance was determined in whole cell, cytoplasm and nucleus fractions (western blot), and mRNA (qPCR) from cells incubated (0.5-8 hours) with insulin. Insulin increased (~1.2 fold, $P<0.05$, ANOVA) pGL3-hENT2-1491 and pGL3-hENT2-1998 activity, but did not alter pGL3-hENT2-1491mut. Insulin (8 hours incubation) also increased Srebp-1c protein abundance and translocation to the nucleus. The Srebp-1c mRNA expression increased by 1 hour, returning to basal values after 2 hours insulin. The results suggest that insulin-increased *SLC29A2* activity requires the Srebp binding consensus sequence including the cytosine at -1129 bp of the promoter region. Support: CONICYT (ACT-73 PIA, AT-24120944, AT-24120941, AT-24120940), FONDECYT (1110977, 11110059). EG-G, PA and CS hold CONICYT-PhD fellowships. CS and PA hold Faculty of Medicine, PUC-PhD fellowships.

111.Transcriptional profiles of ripening genes under methyl jasmonate (MeJA) treatment in *Fragaria chiloensis* fruit. **Figueroa, C.R.**[1,2], Schwab, W.[3]. Facultad de Ciencias Forestales, Universidad de Concepción, Concepción, Chile.[1], Centro de Biotecnología, Universidad de Concepción, Concepción, Chile.[2], Biotechnology of Natural Products, Technical University München, Freising, Germany.[3].

The initiation of gene expression changes that contribute to fruit ripening is mostly triggered by plant hormones. In this sense, the signaling compound methyl jasmonate (MeJA) may appear as possible trigger signal of some relevant aspects of fruit ripening. By means of Real-Time quantitative PCR (RT-qPCR) the transcription levels of several ripening genes were determined in Chilean strawberry (*Fragaria chiloensis*) fruit treated with 10 and 100 uM MeJA during 9 days. The effects of MeJA on transcriptional profiles of genes related with phenylpropanoid pathway; jasmonates and ethylene biosynthesis; and allergens were analyzed. The phenylalanine ammonia lyase 1 (PAL1) and trans-cinnamate 4-monooxygenase (C4H) genes were activated under MeJA treatment. About the lignin biosynthesis genes, MeJA activates the expression of cinnamyl alcohol dehydrogenase (CAD) gene at 2 d and peroxidase 27 (POD27) gene at 9 d. Under the 100 uM MeJA treatment, the 12-oxophytodienoate reductase 3 (OPR3) gene was notably induced at 5 d. In the case of ethylene biosynthesis, an induction of expression of 1-aminocyclopropane-1-carboxylate oxidase (ACO) and 1-aminocyclopropane-1-carboxylate synthase (ACS) genes was observed with 100 uM MeJA, indicating a possible activation of ethylene synthesis by MeJA. In Fra a allergens, an induction of MeJA of gene expression was observed in all those genes at 2 d of MeJA treatments. These results suggest that MeJA could have an important role in regulating some specific events of fruit ripening. Work funded by FONDECYT 11110171 and DAAD scholarship A/11/00747.

112.The role of Ca2+ in the nitrate signaling pathway in Arabidopsis thaliana roots. **Riveras, E.**[1], Oses, C.[1], Tamayo, K.[1], Gutiérrez, R.[1]. Center for Genome Regulation. Millennium Nucleus Center for Plant Functional Genomics. Departamento de Genética Molecular y Microbiología. P. Universidad Católica de Chile.[1].

Nitrate is the main nitrogen source in agriculture soils. Besides its role as a nutrient, nitrate act as a signal that control the global gene expression in plants. However, the signal transduction pathway involved in the nitrate response still remains elusive. It is known that calcium is an essential second messenger in signal transduction in plants. Nonetheless, its role mediating the response to nitrate has not been addressed. As a first criterion to determine if calcium is involved in the nitrate response, we tested whether nitrate produced an increased in cytoplasmic calcium concentration. Using calcium reporter lines, we demonstrated that nitrate treatments produce a quick and strong increase in cytoplasmic calcium. Furthermore, the response of sentinel nitrate responding genes in Arabidopsis roots is altered in the presence of calcium channel blockers and calcium chelators. To identify factor that mediate change in cytoplasmic calcium levels, we evaluate the contribution of phospholipase C in the nitrate response. Using a pharmacological inhibitor of phospholipase C (PLC), we determined that phospholipase C (PLC) is involved in the regulation of sentinel nitrate responding genes. We also found that nitrate treatments increase inositol (1, 4, 5) triphosphate (IP3) levels, the product of PLC activity. Taken together, these results strongly suggest that phospholipase C increase IP3 and intracellular Ca2+ levels leading to changes in gene expression in response to nitrate. Acknowledgment: MilenioP10-062-F, Fondap1509007, Fondecyt110698, HHMI, Beca AT-24121649 CONICYT.

113.Expression profiles of grapevine genes coding for putative BOR efflux transporters **Roa, R.**[1], Pérez-Castro, R.[2], González, S.[1]. Instituto de Biología Vegetal y Biotecnología, Universidad de Talca[1], Laboratorio de Investigaciones Biomédicas, Facultad de Medicina, Universidad Católica del Maule[2].

Boron (B) is an essential micronutrient for normal development of vegetative and reproductive tissues in plants. Due to its role in the structure of rhamnogalacturonan II, a polysaccharide required for pollen tube growth, B deficiency has been associated with the occurrence of parthenocarpic seedless grapes in some varieties of Vitis vinifera L. A narrow range of B concentrations is favorable for plant development. Outside of this range, B can be toxic or can trigger deficiency symptoms. In Arabidopsis plants grown under B limiting conditions, boron is incorporated from the soil and relocated to aerial tissues by xylem loading through the efflux transporter AtBOR1, while tolerance to toxic B concentration is mediated by the activity of the AtBOR4 protein. In grapevine, six putative genes coding for BOR transporters were identified by genome analysis. When predicted proteins were compared with Arabidopsis BOR transporters, three of them were found to be similar to AtBOR1 protein while the other three share similarity with AtBOR4 transporter. The transcriptional pattern of the putative grapevine BOR coding genes in vegetative and reproductive tissues was assessed by qPCR. A differential expression profile was determined for these genes in both, vegetative and reproductive tissues at different developmental stages, as well as in response to exogenous treatments with hormones, elicitor compounds and sodium borate, suggesting different roles for the encoded transporters in B assimilation and distribution in grapevine plants.Financial Support: Fondecyt 1120871.

114.The NE1 scaffold protein regulates Arabidopsis nitrogen use efficiency and plant growth. **Vidal, E.A.**[1,2,3], Araus, V.[1,2,3], Puelma, T.[1,2,3], Gutiérrez, R.A.[1,2,3]. FONDAP Center for Genome Regulation[1], Millennium Nucleus Center for Plant Functional Genomics[2], Departamento de Genetica Molecular y Microbiologia, Pontificia Universidad Catolica de Chile[3].

Nitrogen (N) is an essential macronutrient and a key factor that regulates plant growth and productivity. The increasing demand of agricultural production has led to massive N-based fertilizer use worldwide that involves both high costs for the agricultural sectors and major detrimental effects on the environment. Considering that world population and food needs will continue to grow, it is compelling for modern agriculture to improve N use efficiency (NUE). However, little is known about the molecular factors and signalling pathways controlling NUE in plants. In order to predict genes involved in controlling NUE in Arabidopsis, we used Discriminative Local Subspaces (DLS), a novel bioinformatics method that combines supervised machine learning and coexpression data to predict genes involved in biological processes of interest. Among the genes identified by DLS, the scaffold protein NE1 was the top ranked prediction. We found that a NE1 overexpressor line had diminished NUE, shorter primary roots and less biomass than wild-type plants when grown in limiting N conditions, suggesting that NE1 is a negative regulator of plant NUE and growth and that its effect depends on external nitrate availability. To determine possible targets of NE1, we performed transcriptomics analysis of overexpressor and wild-type plants grown on limiting N conditions. Among the regulated genes we found the nitrate transporter *NRT2.1* and the glutamine synthetase gene *GLN2*, indicating that NE1 partly acts by controlling nitrate acquisition and assimilation in Arabidopsis.Acknowledgements: International-Early-Career-Scientist-program-HHMI, FONDAP CGR-15090007, Millennium-Nucleus-Center-PFG-P10-062-F, FONDECYT-1100698, CONICYT-ANR-07.

115.Preferential retention, expression and function of phytoene synthase (*PSY*) genes during the evolution of the *Brassiceae*
Federico, M.L.[1], Arriagada, A.[1], López, A.[1,2], Gajardo, H.[1], Iñiguez-Luy, F.[1]. Centro de Genómica Nutricional Agroacuícola_CGNA, Unidad de Genómica y Bioinformática, Temuco, Chile.[1], Universidad de Talca, Programa de Doctorado en Cs. Mención Ingeniería Genética Vegetal, Talca, Chile.[2]. Sponsored by Norambuena, L.

Phytoene synthase (PSY) has been shown to catalyze the first committed and rate-limiting step of carotenogenesis in several crop species, including *Brassica napus*. Due to its pivotal role, PSY has been a prime target for breeding and metabolic engineering the carotenoid content of seeds, tubers, fruits and flowers. In *Arabidopsis thaliana*, PSY is encoded by a single copy gene. We have recently shown, however, that *PSY* genes have been retained in a triplicated state in the A- and C- Brassica genomes, with each paralog mapping to syntenic locations in each of the three *Arabidopsis-like* subgenomes. We have also demonstrated that all Brassica *PSY* homologues are expressed, exhibiting overlapping redundancy and signs of subfunctionalization among photosynthetic and non photosynthetic tissues. This later evidence supports the hypothesis that functional divergence of *PSY* gene expression facilitates the accumulation of high levels of carotenoids in non-photosynthetic tissues. To further investigate the reasons behind the preferential retention of Brassica *PSY* genes in spite of the well documented fractionation of Brassica subgenomes, we have determined and compared the gene retention levels of a group of *PSY* neighboring genes and 14 other carotenoid biosynthetic genes using comparative genomics with *A. thaliana*. In addition, we have explored the level of interconnectivity of *PSY* paralogs with other enzymes of the carotenoid and other core metabolic pathways by looking at co-expression levels in different plant tissues.This research was funded by FONDECYT 1090726 and CONICYT REGIONAL/GORE LA ARAUCANIA/CGNA/R10C1001.

116.Expression profiles of members from a gene family coding for zinc-uptake transporters in grapevine. **González, S.**[1], Gaínza-Cortés, F.[2], Pérez-Díaz, R.[1], Ruiz-Lara, S.[1], González, E.[1]. Instituto de Biología Vegetal y Biotecnología, Universidad de Talca[1], Centro de Estudios Avanzados en Fruticultura (CEAF), Conicyt Regional R08I1001[2].

Zinc is an essential micronutrient in plants that plays many important roles in various physiological and metabolic processes, being zinc deficiency one of the most widespread mineral nutritional problems that affect normal development. In grapevine, Zn is required for normal fruit development and deficit of this micronutrient results in the development of clusters containing few berries that also vary in size from normal to very small seedless grains. Because Zn cannot passively diffuse across cell membranes, it must be transported into intracellular compartments for all biological processes where Zn is required. ZIP transporters (Zinc-regulated transporters, Iron-regulated transporter-like Protein), involved in metal uptake and transport have been described in several plants. A gene family composed of 20 members coding for these transporters has been identified in grapevine. The in silico analysis of their promoter regions indicates particular arrays of cis regulatory elements for each gene. A differential expression profile was determined for these genes in both, vegetative and reproductive tissues at different developmental stages. The grapevine *ZIP* genes also shows a different transcriptional pattern in both leaves and fruits exposed to exogenous treatments with hormones, elicitor compounds and zinc chloride.Taken together these results strongly suggest a different role for each gene in maintaining zinc homeostasis along grapevine developmentFinancial Support: Fondecyt 1120871.

117.Ethylene production in fruit of raspberry (*Rubus idaeus* CV. Heritage). **Monsalve, L.**[1], Quiroga, E.[2], Robledo , P.[3], Ayala, A.[2], Martínez, J.P.[2], González, M.[3], Mejia, N.[3], Defilippi, B.[3], Fuentes, L.[4]. Pontifia Universidad Católica de Valparaíso, Valparaíso, Chile[1], INIA La Cruz, La Cruz, Valparaíso, Chile[2], Unidad de Postcosecha, INIA La Platina, Santiago, Chile[3], Centro Regional de Estudio en Alimentos y Salud (CREAS), Valparaíso, Chile[4]. Sponsored by Figueroa, C..
Raspberry (*Rubus idaeus*) is an economic important and a non-climacteric fruit similar to strawberry. Nevertheless, drupelet and receptacles can produce ethylene, but clearly in less amount than classical climacteric fruit. Ethylene is a key hormone to regulate many ripening processes such as softening and flavour development of fruit. To elucidate ethylene production during ripening of raspberry fruit (*Rubus idaeus* CV. Heritage), the respiratory and ethylene rates were tested during maturity and ripening considering both fruit tissues separately: drupelet and receptacles. In addition, transcript levels for ethylene biosynthesis genes, 1-aminocyclopropane-1-carboxylic acid synthase (ACS) and 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) were evaluated in drupelets, receptacles, flower and leaves tissues. Respiration and ethylene rate, ACO and ACS transcript increase during ripening of raspberry fruit. The major levels of transcripts were found in red and over ripening stage and the minor levels of transcripts were observed in green fruit, leaves and flowers. In addition, ethylene rate, ACS and ACO transcripts were higher in receptacle tissue. This information suggests that receptacle has the biosynthetic pathway of ethylene and this tissue should be playing a regulating role on ripening of raspberry fruit.Acknowledge: Proyecto FONDECYT de Iniciación 11110438, "Programa CONICYT de Centros Regionales (Proyecto No.R06i1004). Key words: raspberry, ripening, ethylene biosynthesis.

118.UV-induced transcriptomic response of human keratinocytes treated with aqueous extract of *Deschampsia antarctica*. **García, H.**[1], Bizama, C.[1], Osorio, J.[1], Gutiérrez, A.[1], Gidekel, M.[1]. Uxmal S.A[1]. Sponsored by Ana, G..
Antarctic hair grass (*Deschampsia antarctica* desv; *Poceae*) is the only *Gramineae* that tolerates harsh stress conditions generated by UV irradiation that is present in the Antarctic territory. This particular trait makes of Antarctic hair grass an ideal candidate for extracting new natural active compounds with beneficial biological properties associated to skin protection. On this study we performed a gene expression analysis of human keratinocyte cell lines (HaCaT) exposed to UV irradiation, previously treated with the aqueous extract of *D.antarctica* (AEDA). First, we performed an *in Vitro* study of the photoprotective effect of the AEDA on cells that were irradiated with UV light. Then, by conducting a microarray analysis we were able to identify the genetic expression profiles of cells treated and exposed to a high UV irradiation, and non-treated control. Results showed that the extract presented a dose-dependant photoprotective effect. By using microarray technology we identified differential expressed genes on HaCaT cells previously treated with AEDA and exposed to UV irradiation. These genes are related to biological properties of interest such as regulation of apoptosis, DNA repair, extracellular matrix, and cellular stress response. This study helps identify the main biological functions involved in the UV-induced response of human keratinocytes treated with AEDA. We propose that the aqueous extract of D.antarctica can be of interest in the dermocosmetic area, in the production of skin products that prevent the damage caused by high UV irradiation.

119.ROC-1 is a bZIP transcription factor involved in clock-controlled developmental transitions in *Neurospora crassa*. **Becerra, F.**[1], Montenegro-Montero, A.[1], Weirauch, M.T.[2], Yang, A.[2], Hughes, T.R.[2], Larrondo, L.F.[1]. Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile[1], Banting and Best Department of Medical Research, Donnelly Centre for Cellular and Biomolecular Research, University of Toronto[2].
The generation of asexual spores (conidiation) is an essential means of dispersal among filamentous fungi. In Neurospora, this process is induced by a variety of environmental conditions and is further regulated by the circadian clock. The identification of a strain that enabled clear visualization of circadianly controlled spore formation, known as *band* (due to a mutation in *ras-1*), has been an important tool for the study of circadian rhythms. Under constant conditions *ras-1^{bd}* strains display rhythmic conidiation, while those containing the *ras-1* WT allele do not, although both strains have a functional clock. The molecular mechanisms underlying this phenomenon are largely unknown. We have identified a transcription factor mutant strain that displays rhythmic conidiation in an otherwise WT background. The corresponding gene, dubbed *repressor of conidiation-1*, encodes for a putative bZIP transcription factor. We report that ROC-1 is indeed a sequence-specific DNA-binding protein. We analyzed its DNA-binding specificity by using protein-binding microarrays (PBM), containing all possible 10-base sequences and then scanned the genome with the resulting motif to predict possible targets. Despite its participation in a clock-controlled process, *roc-1* does not itself display a rhythmic expression profile. At present, we are evaluating its general role in conidiation and characterizing its conidial banding under different entrainment regimes and genetic backgrounds. The identification and characterization of ROC-1-associated mechanisms will help understand the complex regulatory events involved in this important circadianly-regulated developmental process and further describe transcriptional networks in this model eukaryote. FONDECYT-1090513.

120.Cytokinin signaling and nitrate induced root growth in *Arabidopsis thaliana*. Naulin, P.[1], **Tamayo, K.**[1], Gras, D.[1], Vega, A.[1], De la Cruz, J.[1], Gutiérrez, R.[1]. Center for Genome Regulation. Millennium Nucleus Center for Plant Functional Genomics. Departamento de Genética Molecular y Microbiología. P. Universidad Católica de Chile.[1].
Nitrate can act as a potent signal to control gene expression, growth and development in plants. However, the mechanisms by which nitrate exert its signaling role to control developmental processes are still poorly understood. We show that nitrate stimulates primary root growth mainly by stimulating meristem activity via cytokinin signaling. Citokinin perception and biosynthesis mutants exhibited shorter roots when grown with nitrate as the only nitrogen source. Histological analysis of the root tip revealed decreased cell division and elongation in the ahk2/ahk4 double mutants as compared to wild-type plants under nitrate regime. Cytokinin perception mutants and wild-type plants grown under nitrate conditions are indistinguishable at day 4 both at the molecular and histological level. However, 10 days after germination there is no detectable meristem activity in the ahk2/ahk4 double mutant and global analysis of gene expression revealed large changes in gene expression in the ahk2/ahk4 double mutant as compared to wild-type plants. Transcriptomics analysis identified important core cell cycle genes that may explain the observed phenotypes. Our results provide strong evidence linking nitrate and cytokinin signaling for the control of active cell division and elongation in the root tip under nitrate conditions. Acknowledgment: FONDAP 15090007, FONDECYT 1100698, NMGFP-P10-062-F, ANR-CONICYT07, HHMI.

121.Effect of methyl jasmonate on ripening and expression of pectate lyase and endoglucanase genes in *Fragaria chiloensis* fruit. Concha, C.[1,2], Oñate, F.[1,2], Figueroa, N.[1,2], Figueroa , C.R.[1,2]. Facultad de Ciencias Forestales, Universidad de Concepción, Concepción, Chile [1], Centro de Biotecnología, Universidad de Concepción, Concepción, Chile[2].

Hormonal regulation of ripening in non-climacteric fruits is not well understood. The plant hormone methyl jasmonate (MeJA) could play a specific role in this process. In this research, we report the effect of MeJA on ripening parameters and expression of the genes encoding pectate lyase (PL) and endoglucanase (EG) during the development of Chilean strawberry (*Fragaria chiloensis*) fruit in an *in vitro* system. Unripe fruits were grown in 10 and 100 uM MeJA during 9 days, and samples were taken at 2, 5 and 9 d after introduction. Effects of MeJA on fruit weight, firmness and chlorophyll *a* and *b* were not significantly different with respect to the control fruit. However, the highest anthocyanin accumulation was found in fruits with 100 uM MeJA treatment at 5 d correlating with an increase in a^* parameter of color measurements in those fruits. On the other hand, MeJA increased the expression of PL and EG genes at 2 d. These genes, according to previous studies of our research group, have MeJA responsive elements in their promoter regions. In conclusion, MeJA could be a hormonal regulator of specific developmental events during ripening of *F. chiloensis* fruit. Work funded by FONDECYT 11110171.

122.Global Search of Novel Transcriptional Regulators involved in the *Neurospora crassa* Circadian Clock. Muñoz-Guzmán, F.[1], Caballero, V.[1], Larrondo, L.F.[1]. Depto. Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.[1].

Circadian clocks are endogenous machineries present in a wide range of organisms and sustained by a cellular autonomous molecular circuit or core oscillator. The basic molecular architecture of these circuits is conserved across evolutionary lineages, and consists on a positive element capable of activating the expression of a negative element that then represses the action of the positive element -inhibiting its own expression. Recent studies in mammals, *Drosophila* and *Arabidopsis* have identified new transcriptional regulators capable of modulating the core oscillator. In *Neurospora crassa* the core elements of the central clock circuit are well characterized but the participation of other transcriptional components in this system is still unknown. In our lab we are trying to identify novel transcriptional regulators affecting the circadian clock in *Neurospora*. To find them, we have started a global search by evaluating a luciferase circadian reporter in KO strains for transcriptional components, spanning a list of close to 200 mutants. We have already found several genes which absence impacts the expression of our circadian reporter. Now we are trying to confirm these alterations by phenotypic analyses and to define how these new components affect the circadian clock. Our results open up the possibility of novel transcriptional loops participating in the control of the *Neurospora* clock, providing important information on the central oscillator and the connecting transcriptional output pathways. Fondecyt 1090513. Conicyt.

123.Spatio-temporal transcriptomic responses to nitrate in Arabidopsis roots. Contreras-López, O.[1,2,3], Vidal, E.A.[1,2,3], Moyano, T.C.[1,2,3], Gutiérrez, R.A.[1,2,3]. Center for Genome Regulation. [1], Millennium Nucleus Center for Plant Functional Genomics. [2], Departamento de Genética Molecular y Microbiología. Pontificia Universidad Católica de Chile.[3].

Genome-wide transcriptional analyses have provided an impressive catalog of N-responsive genes participating in a wide range of processes. Despite this solid groundwork, the molecular mechanisms involved in regulating and coordinating N-responses at the organism, organ or cellular level are largely unknown. In addition, the majority of these genome-wide studies were performed at defined time points in whole plants or organs impairing our understanding of cell-specific regulatory gene networks and how they interact to coordinate organ responses over time. In this research, we map and characterize dynamic N-regulatory networks acting within and/or between cell types in Arabidopsis roots. Our goal is to understand how cell-specific genome-wide responses are orchestrated to produce coherent organ responses over space and time under nitrate treatments. To address this question, we combined cell-sorting, transcriptomics analysis and integrative network bioinformatics to identify cell type-specific regulatory gene networks controlling root responses to nitrate over time. We have been able to characterize N-responsive genes with specific regulation patterns at cell-type level over time. This analysis revealed many N-responsive genes specifically modulated in a spatial or temporal manner, with tissue specific gene clusters showing significant enrichment for different gene ontology terms. These findings suggest a fine tune control over gene responses to N at both cell-type and temporal levels. Milenio P10-062-F, Fondap1509007, Fondecyt110698, HHMI, Beca Apoyo Tesis 24121433, ANR-007 and CONICYT doctoral fellowship grants.

124.Evolution of the roles of the canonical Wnt and BMP pathways during vertebrate osteogenesis: lessons from the amphibian *Xenopus tropicalis*. Bertin, A.[1], Hanna, P.[1], Aldea, D.[1], Otárola, G.[1], Muñoz, D.[1], Sachs, L.[2], Buisine, N.[2], Henríquez, J.P.[1], Marcellini, S.[1]. Faculty of Biological Sciences, University of Concepción, Chile[1], Muséum National d'Histoire Naturelle,CNRS, France[2]. Sponsored by Gutiérrez, J.L.

The Smad-dependent BMP pathway and the canonical Wnt pathway are known to contribute to osteogenesis in mammals. However, our understanding of their epistatic relationship and of their function during skeletal development of other vertebrate groups remains scarce. Here, we describe for the first time the expression of regulators of the canonical Wnt and BMP pathways in osteoblasts and osteocytes of the amphibian *Xenopus tropicalis*. For this purpose, we compared the RNA-seq profiles of undifferentiated osteogenic mesenchyme and of frontoparietal bone (calvaria) containing osteoblasts and osteocytes embedded within the mineralized matrix. Expression patterns were confirmed by RT-PCR, in situ hybridizations and immunohistochemistry on developing skeletal elements and primary cultures of osteoblasts. We provide evidence that positive and negative regulators of Wnt and BMP signaling display dynamic expression patterns as *Xenopus* skeletal cells differentiate. The comparison of our results with data available for mammals reveals both conservation and variation in the expression of key regulators of the canonical Wnt and BMP signaling, such as Sclerostin, Dkk1, Wnt1, BMP5, Noggin1, Noggin2, Gremlin and Endofin. Gain and loss of function experiments in primary cultures of *Xenopus* osteoblasts will represent a powerful mean to assess the functional evolution of the Wnt and BMP pathways during vertebrate skeletal development. Funding: FONDECYT 1110756.

125.Functional analysis of VvMYB4-like, a putative transcriptional repressor of flavonoids. **Pérez-Díaz, R.**[1], Pérez-Díaz, J.[1], Madrid-Espinoza, J.[1], González, E.[1], Ruiz-Lara, S.[1]. Instituto de Biología Vegetal y Biotecnología, Universidad de Talca, Casilla 747, Talca, Chile [1].

Flavonoids are secondary metabolites widely distributed in plants, playing an important role in several biological functions. They confer pigmentation to flowers and fruits, can protect plants against UV damage, diseases and herbivores or be involved in physiological functions such as seed dormancy and viability. In grapevine, anthocyanins and proanthocyanidins are the main flavonoids in berries, which are associated to organoleptic properties in red wine such as color and astringency. Flavonoid pathway is specifically regulated at transcriptional level and several R2R3 MYB proteins have shown to act as positive regulators. However, some members of this family have shown to repress the flavonoid biosynthesis. In this work, we present the partial characterization of *VvMYB4-like* gene, which encodes a putative transcriptional repressor in grapevine. Transient expression of a *VvMYB4-GFP* fusion construct indicates that this protein is localized in the nucleus. Expression analysis in grapevine shows that *VvMYB4-like* mRNA is highly accumulated in leaves and skin berries. To gain further insight into the role of this gene, we generated transgenic plants of Arabidopsis thaliana carrying the construction Pro35S::*VvMYB4-like*. Transcriptional analysis using qPCR of some specific genes of the flavonoid pathway revealed that in all Arabidopsis transgenic lines overexpressing *VvMYB4-like*, the expression of *AtLDOX* is highly down-regulated compared to wild-type plants. Taking together, this data strongly suggest that *VvMYB4-like* is down-regulating flavonoid biosynthesis in grapevine. Financial Support: Genome-Chile Program, Project FONDEF G07I-1003.

126.Effect of oxidative stress on the vernalization response using several *Arabidopsis* ecotypes that express the *FRI/FLC* module. **Moraga, F.**[1], León, G.[1]. Laboratorio de Desarrollo y Reproducción de Plantas, Centro de Biotecnología Vegetal, Universidad Andrés Bello [1].

Flowering in plants is under a tight genetic and environmental control, and the most important pathways regulating this process are the photoperiod and vernalization. Both converge in the master regulatory gene *FLOWERING LOCUS T (FT)*, which encodes a small protein produced in the leaf vasculature that travels to the shoot apical meristem, activating the expression of the floral meristem identity genes. FRIGIDA repress flowering through the transcriptional activation of *FLOWERING LOCUS C (FLC)*, which encodes a MADS-Box protein that repress *FT* expression. During vernalization, *FLC* promoter is remodeled and the gene becomes transcriptionally silenced, allowing *FT* expression. Most laboratory strains of Arabidopsis, as Col-0, do not have a functional *FRI/FLC* module. In this work we have used several northern Arabidopsis strains that express high levels of *FLC* and thus have cold requirements to flowering. We?ve used these plants to determine the amount of cold that is necessary to flowering and found that mild oxidative stress could compensate sub-optimal cold treatments. *proFLC:GUS* transgenic plants show a reduction in GUS histochemical activity when grown on salicylic acid (SA), which indicate that this response is controlled at the transcriptional level. Indeed using qPCR, we found that levels of *FLC* transcripts decreased after the treatment with SA, allowing *FT* expression. Taken together our results suggest that northern accessions of Arabidopsis are an interesting model to study cold requirements and that mild oxidative stress could promote flowering through inactivation of *FLC*. Funded by Fondecyt1120766 and UNAB DI-23/10-R.

127.Effects of UV-B radiation on myb-mediated transcriptional regulation of flavonoid biosynthetic pathway in grapevine (*Vitis vinifera*. L) leaves. **Loyola, R.**[1], Matus, J.T.[2], Arce-Johnson, P.[3]. Departamento de Fruticultura y Enología. Facultad de Agronomía e Ingeniería Forestal. Pontificia Universidad Católica de Chile, Santiago, Chile[1], CRAG-Centre de Recerca Agrigenomica, CONSORCIO CSIC-IRTA-UAB, Barcelona, Spain.[2], Departamento de Genética Molecular y Microbiología. Facultad de Ciencias Biológicas. Pontificia Universidad Católica de Chile, Santiago, Chile.[3]. Sponsored by Arce-Johnson, P.

Abstract: Ultraviolet-B radiation (UV-B, 280-315 nm) is a natural component of sunlight and a key environmental signal that is specifically perceived by plants to promote UV acclimation and survival to solar radiation. Some reports have analyzed the changes in gene expression triggered by UV-B light on several species, including grapevine, using microarray technology. However, there is no information on MYB-mediated transcriptional regulation of flavonoid biosynthetic pathway in response to UV-B in grapevine. In this study we investigate the gene expression of UV-B responsive genes, by qRT-PCR analysis of leaf from *in vitro* cultured *Vitis vinifera* cv. Cabernet Sauvignon plants exposed for 5 h at ?30 ?W cm-2 fluence rate of artificial UV-B radiation. We present the expression characterization of MYB genes identified by us controlling negatively flavonoid accumulation in grape, such as *VvMYB4a/b* repressors, which are repressed in UV-B+ treatment. Also, the *VvMYBF1*, *VvFLS4* and *VvC4H* genes are induced in UV-B+ treatment. Moreover, the UV-B photoreceptor *VvUVR8* and bZIP transcription factor *VvHY5* genes, which plays a important role in UV-B perception and signaling, respectively, are induced in UV-B+ treatment. The analysis of these genes and their promoters will provide highlights for understand the mechanisms of regulation of phenylpropanoid pathway in response to UV-B radiation. **Acknowledgements**: Fondecyt 1100709, Innova Corfo 07Genoma01, Millennium Nucleus in Plant Functional Genomics P 10062-F.

128.Characterization of promoters from grapevine with different expression profiles to develop cisgenic grapes. **Espinoza, C.**[1], Fredes, I.[1], Herrera, D.[1], Torres, E.[2], Arce, A.[1], Serrano, A.[1], Larraín, R.[1], Marchandón, G.[3], Medina, C.[1], Arce-Johnson, P.[1]. Departamento de Genética Molecular y Microbiología. Facultad de Ciencias Biológicas. Pontificia Universidad Católica de Chile, Santiago, Chile.[1], Facultad de Ciencias. Universidad de Chile, Santiago, Chile.[2], Facultad de Ingeniería. Universidad de Talca, Talca, Chile.[3].

Grapevine is one of the most cultivated crops worldwide and is the most economically relevant crop in Chile. Its genomic sequence has been completed making available new sources of information for genetic manipulation improving grape characteristics by transgenic technology. Limitations to the use of transgenic plants have prompted the development of alternative technologies. Cisgenic approach is a marker-free technology that uses native genes to improve crops properties, but requires knowledge of native promoters to drive gene expression. This work aims the identification of grapevine promoters with a wide range of expression profiles. We have been studied several genes, including VvTMT2, a putative monosaccharide transporter; the metallothionein VvGRIP24; a virus-responsive gene VvNAC1; VvUBI, an ubiquitin coding gene; VvSLP, a sucrose-like protein; VvUG4E, an UDP-glucose-4-epimerase, VvTL2 with unknown function and the transcription factor VvMYBA1. An in silico analysis identifies the putative positive and negative regulatory cis elements present in 2000pb of each promoter. The expression pattern of the mentioned genes was analyzed in different tissues and developmental stages, as well as under several stresses and stimuli, giving a broad range of expression patterns, including genes expressed exclusively during ripening, in response to sugars, senescence, biotic stress, among others; and genes with strong and constitutive expression were also identified. Functional analysis using reporter genes have been conducted in order to confirm promoters transcription activity. This work provides an helpful tool for future genetic breeding in grapevine. Acknowledgement: Fondecyt 1100709, CORFO07GENOMA01, Núcleo Milenio P10062-F.

129.Transcription analysis of insulin-like receptor in the surf clam *Mesodesma donacium*. **Alarcón-Matus, P.**[1], **Valenzuela-Muñoz, V.**[1], **Núñez-Acuña, G.**[1], **Aguilar-Espinoza, A.**[1], **Gallardo-Escárate, C.**[1]. Laboratorio de Biotecnología y genómica acuícola Departamento de Oceanografía, Casilla 160-C Universidad de Concepción Concepción, Chile [1]. Sponsored by Uribe P, E.A.
The insulin receptor in vertebrates has a pivotal role in the signalling pathway activation of metabolic process involved in cellular mitosis and somatic growth. Although insulin has only been reported for vertebrates species, recent findings have identified orthologous sequences of insulin-like receptors (ILR) in invertebrates organisms. The aim of this study was to characterize and evaluate the transcription expression of ILR in the surf clam *Mesodesma donacium* exposed to experimental trials with different levels of feed. To identify the ILR gene, a EST-data set was generated by 454-pyrosequencing and then cloned using specific primers on putative ILRs sequences. Real time PCR was performed in groups of bivalvesdaily fed with *Isochrysis galbana* microalgae (1 million cell/mL) and without food during 30 days. With respect to the molecular characterization of *M. donacium* ILR gene, the sequence revealed a partial ORF of 767 bp. This sequence was highly similar to orthologous sequences reported for insects. Further, qPCR analysis showed that digestive gland tissue had the higher expression level over other tissues such as mantle, foot, gonad and gill. The individuals daily fed had also the higher levels of ILR expression. The results obtained in this study support the hypothesis that the ILR can be associated with somatic growth in marine invertebrates and therefore be applied as a potential DNA marker in aquaculture.Funding Fondecyt 1120397.

130.Unraveling the spinal cord regeneration transcriptome of *Xenopus laevis*. **Almonacid, L.I.**[1,2], Lee-Liu, D.[3,4], Moreno, M.[3], Melo, F.[1,2], Larrain, J.[3]. Molecular Bioinformatics Laboratory, Millennium Institute on Immunology and Immunotherapy[1], Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Alameda 340, Santiago, Chile[2], Center for Aging and Regeneration and Millennium Nucleus in regenerative Biology[3], Faculty of Chemical and Pharmaceutical Sciences, Universidad de Chile, Santiago, Chile[4].
It is widely known that some organisms have the ability to regenerate whole body parts. Although this capability is fascinating, its study can be complex and overwhelming due to the variety of involved tissues, considering that not all tissues respond in the same fashion. In order to provide a richer and focused view of the regeneration process, we performed high-throughput mRNA sequencing to study the spinal cord regeneration in *Xenopus laevis*. Regenerative (R) and non-regenerative (NR) stages were defined and within each, three measures were performed at different times after injury. Our results allowed us to characterize up to twenty-seven thousand transcripts. About 2,000 of them showed a clearly distinct expression pattern. In the R stage a gene ontology analysis showed genes related to the cell cycle consistent with the fact that, after injury, new cells must replace those that have been damaged. The NR stage showed an activity of transcripts related to immune response and response to stress. We currently are validating and characterizing individual genes that may have a key role in spinal cord regeneration.

131.Nitrogen gene networks and plant defense response to pathogen attack. **Vega , A.**[1,2], Gutiérrez, R.A.[1,3]. Center for Genome Regulation. Millennium Nucleus Center for Plant Functional Genomics. [1], Departamento de Ciencias Vegetales. Facultad de Agronomía e Ingeniería Forestal. Pontificia Universidad Católica de Chile[2], Departamento de Genética Molecular y Microbiología. Pontificia Universidad Católica de Chile. [3].
Nitrogen (N) is a primary limiting factor for plant growth and yield in agriculture. N is an essential macronutrient with profound effects in different developmental programs in plants. It is well known that nitrate and other N metabolites affect root system architecture, leaf growth and flowering time, among other plant developmental processes. In addition, the plant N nutritional status influences its ability to respond effectively when challenged with plant pathogens. Although significant progress has been made in the identification and characterization of defense-response genes, the molecular mechanism, signaling and the regulatory networks involved in the interaction between N and pathogen responses are poorly understood. To answer this question, we evaluated *Arabidopsis thaliana* defense response to the bacterium *Pseudomonas syringae* under contrasting nitrogen regimes. N availability alters Arabidopsis susceptibility to this bacterial pathogen. To identify candidate regulatory gene networks mediating the plant defense response associated with nitrogen nutrition, we used GENIUS a new machine learning algorithm developed in our group to infer functional gene networks. Experiments are underway to validate key components of both N regulation and the defense response in Arabidopsis that were identified with our bioinformatics approach. We expect to further expand these results in Tomato, an agronomic plant. These results are contributing to a better understanding of the convergence points between biotic stress and N status and metabolic regulation in *Arabidopsis thaliana*. Acknowledgements: Fondecyt-11110095, International-Early-Career-Scientist-program-HHMI, FONDAP CGR-15090007, Millennium-Nucleus-Center-PFG-P10-062-F.

132.Ferritin characterization and identification of single nucleotide polymorphism (SNP) associated with innate immune response *Concholepas concholepas*. **Chávez - Mardones, J.**[1], Nuñez - Acuña, G.[1], Maldonado - Aguayo , W.[1], Valenzuela - Muñoz, V.[1], Gallardo - Escárate, C.[1]. Laboratorio de Biotecnología y Genómica Acuícola, Departamento de Oceanografía, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción. P.O. Box 160-C. Concepción, Chile.[1]. Sponsored by Uribe P., E.A.
Ferritin is the main iron storage protein at cellular level, participating in the innate immune system through the control of oxidative stress. The aim of this study was to characterize the ferritin gene of Concholepas concholepas (CcFer) and analyze their gene transcription in response to pathogens. Further, single nucleotide polymorphisms (SNPs) within its sequence were evaluated. Herein, specific primers were designed to identify Ferritin from a cDNA pyrosequencing of C. concholepas, and the full mRNA sequences was obtained through RACE approach. To evaluate the immune response of Ccfer, eighteen individuals were exposed to Vibrio anguillarum and measured by qPCR. Finally, SNP genotyping was carried out by High-Resolution Melting (HRM) analysis. The results revealed an ORF of 513pb that encodes to 171 aa. Gene transcription analysis of CcFer indicated that the gill tissue is over-expressed after an exposition to pathogens. Moreover, homozygous mutant genotype (TT) was over-expressed in the group challenged with respect to the wild genotype (CC) and the heterozygous (CT). These results suggest that CcFer is associated with the immune response, and the putative SNP may be involved in the regulation of their transcriptomic levels.Funding FONDEF D091065.

133.Developing methodologies to obtain soluble nuclear matrix protein components associated with vitamin D-mediated transcriptional response in osteoblasts. **Ruiz-Tagle, C.**[1], Montecino, M.[1]. Center for Biomedical Research and FONDAP Center for Genome Regulation, Faculty of Biological Sciences and Faculty of Medicine, Universidad Andres Bello, Santiago, Chile.[1].
The eukaryotic nucleus is organized in multiple structural and functional compartments that concentrate regulatory machineries that regulate critical cellular events, including gene transcription, DNA replication and DNA repair, among others. Our group has recently established that binding of the vitamin D receptor (VDR) to the nuclear matrix (NM) fraction is stimulated by the presence of the ligand 1alpha,25-dihydroxy vitamin D3 (vitamin D). This increased association is reflected by a punctuate distribution within the nucleus and does not involve the Runx2 protein, a transcription factor previously shown to be highly enriched in the NM and capable of interacting with VDR in osteoblasts. Therefore, there is a requirement for establishing the mechanism by which VDR is retained at the NM. In particular, it becomes relevant to identify NM protein partners that may account for VDR association following ligand stimulation. Most of the currently available procedures for NM isolation result in highly insoluble proteins, thus limiting their identification and characterization based on functional protein-protein interaction assays. Here, we described a methodology that allows isolation of soluble and intact NM proteins from human osteoblasts. We demonstrate that our protocol yields high quality NM proteins that can be easily applied in GST-pull down assays using VDR as bait. CONICYT 24120956, UNAB DI-72-12/I, FONDECYT 1095075, FONDAP 15090007.

134.HMGA1a protein positively stimulates the binding of estrogen receptor and its effect on the chromatin remodeling complex SWI/SNF. **Del Rio, V.**[1], Hepp, M.I.[1], Gutiérrez, J.[1]. Laboratorio de Regulación Transcripcional, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción.[1].
Gene transcription is a highly regulated process in which ATP-dependent chromatin remodeling complexes play a major role. It is known that recruitment of these complexes to specific genomic loci can be facilitated by transcription factors. In this context, it has been observed that the recruitment to gene regulatory regions of the chromatin remodeling complex SWI/SNF would be favored by small non-histone proteins know as HMG. A number of studies suggest that proteins of the HMGA family would be able to favor nucleosomal disruption, although little is known about how HMGA proteins impact the catalytic activity of SWI/SNF complexes. In order to study the contribution of HMGA proteins to targeted nucleosome remodeling activity of human SWI/SNF we performed in vitro nucleosome remodeling assays, using six different probes (228 bp) reconstituted as mononucleosomes. These probes contain an ERE (Estrogen Receptor Element) and a nucleosome positioning sequence termed 601, differing in sequences designed for HMGA binding. Recombinant HMGA1a and Estrogen Receptor alpha were used in these assays, in addition to affinity purified hSWI/SNF complex. Our results show that hHMGA1a binds preferentially to particular AT-rich sequences. ERα binding is enhanced in the presence of hHMGA1a, although AT-rich sequences did not favor this effect of HMGA1a on ERα. Moreover, we observed that hHMGA1a enhances the ERα-mediated remodeling activity of hSWI/SNF. Our results suggest that hHMGA1a can stimulate the targeted nucleosome remodeling activity of the hSWI/SNF complex. Fondecyt-1085092; DIUC-211.037.014-1.0.

135.Comparisson of two funtional ammonia-lyase from Rhodobacter Sphaeroides (Rs). **Escalona, E.**[1], Guzmán, L.[2], Gentina, J.C.[3], Aguilar, F.[4], Acevedo, W.[4]. Laboratorio de química-biológica, Pontificia Universidad Católica de Valparaíso.[1], Laboratorio de química-biológica, Pontificia Universidad Católica de Valparaíso[2], Laboratorio de cultivos microbianos, Pontificia Universidad Católica de Valparaíso[3], Laboratorio de Bio-Espectroscopía Molecular, Pontificia Universidad Católica de Valparaíso[4]. Sponsored by Guzmán, L.
A putative ammonia-lyase, enzyme codified in the chromosome 1 from the photosynthetic bacterium Rhodobacter sphaeroides (RsTALCI) was identified, cloned and functionally expressed using the pRSET-B vector in Escherichia coli, and compared with the RsTALCII codified in the chromosome 2. RsTALCII is a tyrosine ammonia-lyase (TAL) well characterized until now. The RsTALCII catalyzes the conversion of tyrosine to p-hydroxycinnamic acid (pHCA) a compound with attractive biological activities as: anti-tumoral, lipid-lowering and anti-inflammatory. Amino acid sequence comparison showed a 87% of homology, presenting a similar active site. Xue et al., 2007, showed that RsTAL CII enzyme accept both tyrosine and phenylalanine as substrate. The aim of this research was: 1) characterize the RsTALCII and RsTALCI at biochemical (Km and Vmax) and molecular level; 2) produce pHCA in a bacteria suitable for industrial use. The tal1 and tal2 genes were amplified by PCR from the genomic DNA from Rs and were cloned in the pRSET-B and pBTBX-2 respectively. The protein expression was induced with IPTG and L-arabinose respectively and enzymatic assays were carried on cell extract.Our results showed that: 1) RsTALCII protein have affinity only for tyrosine as substrate to produce pHCA (Km= 0,4 mM) and RsTALCI did not show tyrosine or phenylalanine ammonia-lyase activity. The production of pHCA at flask level was measured by HPLC.Our studies illustrate that Rs contains ammonia-lyase enzymes significantly different. At future the RsTALCI activity will be studed with hystidine as substrate.Acknowledge: PUCV, project 037.430-2012.

136.Similar states of DNA methylation in MLH1 gene are associated with different patterns of histone modifications in different types of cancer cells. **Sepúlveda, J.H.**[1], Gutiérrez, S.E.[1]. Laboratorio de Regulación Transcripcional y Leucemia, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción[1].
MLH1 gene has important functions in DNA repair and it has been shown to be silenced in certain types of cancer. Lack of MLH1 expression results in the accumulation of errors in the cell DNA, facilitating cancer progression. Therefore, it is important to analyze the repressive epigenetic mechanisms involved in the transcriptional repression of this gene.This research seeks to determine the chromatin structure epigenetic marks present at in the MLH1 promoter in two cell lines: HeLa and HL-60. To this end, methylation specific PCR (MSP) was used to evaluate DNA methylation status of the MLH1 promoter and ChIP assays to analyze the level of monomethylation at lysine residues K4, K9 and K27 as well as the trimethylation at lysine residues K4 and K9 histone H3. We find that although both cell lines shown DNA methylation of MLH1 promoter, histone modifications exhibit differential patterns in HeLa and HL-60 cells. HeLa present a high enrichment in H3K4me3 and low enrichment in H3K4me, both marks are associated with transcriptional activation. In contrast, HL-60 exhibit significant enrichment in H3K27me and a lower association of H3K4me3 marks associated with transcriptional repression and activation respectively. These results suggest that MLH1 transcriptional repression is differentially regulated in the two cell lines analyzed. FONDECYT 1100670.

137.Identification of CREB and C/EBPβ transcription factors binding elements in the proximal promoter region of human RIC-8B gene. **Maureira, A.**[1,2], Sánchez, R.[1,2], Hinrichs, M.V.[1,2], Gutiérrez, J.[2,3], Olate, J.[1,2]. Laboratorio de Genética Molecular[1], Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Chile[2], Laboratorio de Regulación Transcripcional[3].

The G protein alpha stimulating subunit ($G_s\alpha$), involved in cAMP signaling, participates in the regulation of many cellular processes which generates pleiotropic effects throughout the whole organism. Such a complex phenomenon may be explained in part by the action of numerous protein modulators with differential tissue- and developmental stage-dependent expression. RIC-8B, a protein which acts as a ligand-independent positive modulator over $G_s\alpha$, shows a restricted tissue mRNA expression pattern in mammals. In order to understand this gene transcriptional regulation, we have previously identified a DNA region with promoter activity, localized upstream to the human RIC-8B gene. Bioinformatic analyses performed on this region revealed the presence of potential response elements for CREB and C/EBP transcription factors, which are confined to the proximal promoter region. Using cell nuclear extracts and EMSA analyses we confirmed the presence of only two CRE sites, but not C/EBPβ binding sites. In our present work, we were able to demonstrate the presence of both, the CRE and C/EBP sites in the proximal human RIC-8B gene promoter, using the purified recombinant C/EBPβ2 and CREB1B human transcription factor isoforms. The differences observed between the two C/EBPβ assays may be due to the low transcription factor protein levels present in the nuclear extracts employed in the aforementioned EMSA assays. Acknowlegments: FONDECYT-1090150; DIUC-211.037.014-1.0.

138.Contribution of sumoylation during regulation of vitamin D-mediated transcription in osteoblastic cells. **Merino, P.**[1], Montecino, M.[1]. Center for Biomedical Research and FONDAP Center for Genome Regulation, Faculty of Biological Sciences and Faculty of Medicine, Universidad Andres Bello, Santiago, Chile.[1].

The vitamin D receptor (VDR) belongs to the superfamily of nuclear receptors and functions as a transcription factor, modulating gene expression in response to its ligand, 1alfa,25-dihydroxy vitamin D3 (Vitamin D). Binding of this ligand to the C-terminal of VDR promotes a conformational change that allows interaction with transcriptional coactivators, including members of the p160/SRC family and DRIP205 subunit of the DRIP/TRAP/Mediator complex. It has been described that interaction of nuclear receptors (e.g. estrogen receptor) with transcriptional coactivators can be modulated by sumoylation, a post-translational modification that occurs at both the receptor and coactivator moieties and which affects transcriptional activity. We have analyzed the aminoacidic sequence of VDR and found putative sumo interacting motifs at its C-terminus. Additionally, potential SUMO-1-acceptor lysine residues were identified at SRC-1 and DRIP205 coactivators. Therefore, here we explore the contribution of sumoylation in the transcriptional regulation of the vitamin D-responsive osteocalcin (OC) gene in osteoblastic cells by combining transient over-expression, RT-PCR, luciferase assays and co-immunoprecipitation studies. We find that endogenous OC gene transcription is decreased by forced expression of the sumoylation machinery components SUMO-1, E1-Ubc9 and E3-Pias-Gama, in the presence of vitamin D. OC expression also diminishes after over-expression of a dominant-negative form of SENP-1, a desumoylase. Together, our results indicate that, following exposure of osteoblastic cells to vitamin D, sumoylation plays an important regulatory role at vitamin D-responsive genes. FONDECYT 1095075, FONDAP 15090007, UNAB DI-198-12/I, CONICYT 24120998.

139.Transcriptome response of *Mytilus galloprovincialis* exposed in vivo to Saxitoxin (STX). **Núñez-Acuña, G.**[1], Aballay, A.E.[2], Astuya, A.P.[2], Gallardo-Escárate, C.[1]. Laboratorio de Biotecnología y Genómica Acuícola, Centro de Biotecnología, Departamento de Oceanografía, Universidad de Concepción, Casilla 160-C, Concepción, Chile.[1], Laboratorio de Genómica Marina y Cultivo Celular, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Casilla 160-C, Concepción, Chile.[2]. Sponsored by Uribe P.E.A.

Saxitoxin (SXT) is one of the main phycotoxins that contribute to paralytic shellfish poisoning. Paralytic shellfish poisoning (PSP) is produced by marine algae and can also be produced by certain bacteria, cyanobacteria and red algae. There is evidence of changes at the physiological level in bivalves exposed to SXT, although this has not been subject to genomic studies. The present work assesses the expression of thirteen candidate genes associated with cellular stress and immune response in the mussel *Mytilus galloprovincialis* exposed to STX. Analysis with qPCR showed overexpression in all the candidate genes in the challenged specimens, with different patterns of expression registered according to their biological function. As well, the expression patterns of some genes were correlated to increased exposure time to STX, generating clusters according to their biological functions. Our results characterize transcripts validated by qPCR at different exposure times to STX, providing valuable information to understand the effects of the interactions between this marine toxin and the innate immune systems of marine invertebrates. Acknowledgement. This work was funded by CONICYT-Chile through FONDECYT project N° 1120397.

140.Mining Single nucleotide polymorphism (SNP) in *Haliotis rufescens* using transcriptome pyrosequencing data. **Valenzuela-Muñoz, V.**[1], Uribe, D.[1], Gallardo-Escárate, C.[1]. Laboratorio de Biotecnología y Genómica Acuícola, Centro de Biotecnología. Departamento de Oceanografia. Universidad de Concepción, Concepción, Chile. [1]. Sponsored by Uribe P. E.A.

One of the main problems to solve in the abalone farming is the low rate growth under cultured conditions. Herein, molecular markers as single nucleotide polymorphisms (SNP), have become the most promised DNA markers due its association with a phenotype of interest. This study reports novel SNP markers for the red abalone *Haliotis rufescens*. From a 454 pyrosequencing based on transcriptome, we performed a De novo assembly by CLC Genomics Workbench software. SNPs were mined with a maximum coverage and variant frequency of 100 and 35% respectively. A total of 622 SNPs were identified in 146 sequences, and an estimated frequency of 1 SNP each 1,000 bp. We selected a total of 10 SNPs markers annotated by GO analysis, 8 SNPs of them were annotated for Heat shock protein 70 (HSP70), vitellogenin (VTG), sperm lysin, Alginate lyase enzyme, Glucose-regulated protein 94 (GRP94), fructose-bisphosphate aldolase, sulfatase 1A precursor and ornithine decarboxylase antizyme (ODC), the remaining 2 sequences have no similarity with others reported in public data base. Also the SNPs markers were validated through High Resolution Melting Analysis (HRMA) and correlated with differential growth rates in farmed abalones. This study provides new SNPs markers for genus Haliotis to be used as genomic tools to improve the Chilean abalone aquaculture.Funding: Fondef D09I1067.

141.Analysis of non-canonical introns in the human transcriptome. Parada, G.[1], Munita, R.[1], Gysling , K.[1]. Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Chile.[1]. Sponsored by Canessa, P.

Intron recognition relies on sequence-specific interactions between the intron terminal regions and the spliceosome ribonucleoprotein particles. Most of introns are of the U2-type with GT-AG dinucleotides at their splice junctions, while a minor group is of the U12-type that may also have AT-AC dinucleotides. At present, there are few cases of non-canonical introns reported. We have done a comprehensive analysis of non-canonical splice junctions using human transcriptomic data generated by the new high-throughput sequencing technologies (RNA-seq). About 1.2 billion of RNA-seq reads from a mixture of 16 human tissues were mapped to the reference genome. In addition, we mapped 1.3 billion of RNA-seq reads from GM12878, a human lymphoblastoid cell line, to their personalized genome. The resulting alignments were processed through a set of stringent filters and then integrated with the available EST/cDNA data. We found 305 non-canonical introns that cannot be explained by genomic polymorphisms at their splice sites. Furthermore, 65.25% of them were not previously annotated by GENCODE. Most of these non-canonical introns have U2/U12-like sequences and participate in alternative splicing. Moreover, we found non-canonical splice junctions that are evolutionary conserved. We validated some of these non-canonical introns by RT-PCR. In conclusion, we report the non-canonical intron landscape of the human transcriptome. Further research is needed to understand how these non-canonical introns are processed by the splicing machinery and to evaluate their impact at human proteome diversity. Supported by grants Fondecyt-1110392 and MSI-P10-063-F.

142.Evaluation of the methylation status of RPRM gene in gastric cancer models. Marchant, M.J.[1,2], Corvalán , A.[2], Guzmán, L.[1]. Instituto de Química, Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso.[1], Centro de Investigaciones Médicas, Pontificia Universidad Católica de Chile.[2].

Gastric cancer (GC) is the leading cause of cancer death worldwide. Therefore, is relevant identify its molecular basis. Recent studies show that Reprimo (RPRM) plays a key role as tumor suppressor gene, causing cell cycle arrest at the G2 to M. It is believed that loss of expression and function of RPRM could be involved in the early stages of GC.RPRM expression was evaluated in GC cell lines: AGS, NCI-N87, SNU-1 and KATO-III by RT-PCR. We verified the methylation status of the promoter region through bisulfite sequencing.We determined that 75% of the lines studied expressed mRNA level RPRM. In addition was found that the rate of methylation (methylated CpG v/s non-methylated) in different cell lines studied was 70%.These results suggest that the loss of RPRM expression could be related with high rate of methylation in the promoter region in some cells line. In the case of NCI-N87, contains high levels of methylation in promoter region, however was not able to silence the expression of RPRM. These findings suggest that small differences in the level methylation of CpGs islets play a critical role in the expression of RPRM. Grant Program Fondecyt 1111014.

143.Differential regulatory role of C/EBPβ isoforms in transcriptional regulation of human albumin gene. Valenzuela, N.[1,2], Castro, A.[2,3], Gutiérrez, J.[1,2]. Laboratorio de Regulación Transcripcional[1], Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile.[2], Laboratorio de Transducción de Señales y Cáncer[3].

C/EBPβ is a transcription factor involved in different cellular processes. In humans, this protein exists as three isoforms, derived from alternative translation of the same mRNA. It has been described that this factor can act as transcriptional activator or repressor, depending on the participant isoform and other nuclear proteins. We revealed the existence of an interaction between these hC/EBPβ isoforms and ACF/CHRAC complex, which belongs to the ISWI (SNF2H) subfamily of chromatin remodeling complexes. In order to establish a functional role for this interaction we decided to study putative target genes under the influence of this interaction. Bibliography and database searches of genes regulated by hC/EBPβ defined the human albumin gene as a good candidate to analyze the effect of this interaction. qRT-PCR analyses of RNA samples obtained from HepG2 cells co-transfected with vectors encoding hC/EBPβ isoforms and hSNF2H showed that these isoforms generate a differential effect on gene expression and that hSNF2H exerts a repressive effect. Furthermore, we analyzed the expression of the albumin gene in HepG2 cells stimulated to differentiation, observing increased expression of this gene. ChIP assays revealed that hSNF2H and hC/EBPβ bind to the albumin gene promoter only in the absence of the stimulator, pointing to a repressive role of these factors on the expression of this gene. The results obtained in this work suggest that C/EBPβ exerts a repressive effect on the albumin gene through recruitment of a SNF2H-related chromatin remodeling complex. Fondecyt-1085092-1120923; DIUC-211.037.014-1.0.

144.Effects of early CPPU treatments in the gene expression of putative cyclins and berry size in *Vitis vinifera*. Jáuregui R., F.[1], Pérez-Donoso, A.[1]. Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile[1]. Sponsored by Vega, A.

Cytokinins promote cell division. These have been associated with increasing fruit size possibly increasing the number of cells, however, the mode of action is not known. In order to understand this mechanism, we want to characterize the effects of pre-floral applications of synthetic cytokinin on the expression of genes related to cell cycle and its relation to berry size in *Vitis vinifera* clusters. During the 2011-12 season, we applied CPPU pre-bloom on Thompson Seedless in an experimental field (no cultural practices). It was verified that CPPU applications generated significant increases in equatorial diameter for various times of sampling, we verified significant decrease in the ratio polar/equatorial (shape) in the berries and we observed significant increases in soluble solids and reduction in titratable acidity both at veraison and at harvest time. Preliminary results show significant increase in pericarp width and number of cells after application of CPPU. In addition, we identified three possible sequences of cyclinD genes in *V. vinifera* with high identity to cyclinD involved on cell cycle. These genes have characteristics domains like LxCxE and Cyclin Box. These were isolated and we verified differential expression in different vegetative tissues of *V. vinifera*. Currently, these genes are being cloned and we are verifying and quantifying their expression after the CPPU treatments. This could explain the changes in number of pericap cells and changes in berry size. Acknowledgement to CONICYT PhD Scholarship and Scholarship Supporting Doctorate Thesis (24121032).

145.Gibberellin oxidase activities in *Bradyrhizobium japonicum* bacteroids. **Méndez, C.**[1], Valdés, E.[1], Sequeira, G.[1], Montanares, M.[1], Rojas, M.C.[1]. Departamento de Química, Facultad de Ciencias, Universidad de Chile. [1].

Some rhizobacteria synthesize gibberellins (GAs), diterpene phytohormones, as part of their symbiotic interaction with host plants. Particularly the soybean (*Glycine max.*) simbiont *Bradyrhizobium japonicum* contains an operon of GA biosynthetic genes that include two cyclase genes involved in *ent*-kaurene biosynthesis, the first commited intermediate of the pathway, plus several P450 monooxygenase genes that codify for the oxidases involved in further reactions to the final 19-gamma10 lactonic GA products. These bacterial genes would be expressed in soybean root nodules in which the microorganism differentiates into a bacteroid, a specialized form able to fix atmospheric nitrogen as well as to produce plant growth promoting substances such as GAs.In this work, several oxidase activities were investigated with radiolabelled intermediates of GA biosynthesis as substrates in a bacteroid fraction obtained from soybean nodules. Conversion of the substrates into more oxidized products was demonstrated by gas chromatography-mass spectrometry after isolation of the labelled products by partition, reverse-phase chomatography and HPLC. GA oxidase activities in the bacteroids were found to be dependent on the growth stage of soybean plants and was highest at flowering. In contrast to the bacteroid fraction, liquid cultures of *B. japonicum* did not metabolize any of the precursors. The expression of two GA biosynthetic genes was investigated both in liquid cultures and in the bacteroid fraction. Financement: FONDECYT 1110127.

146.Molecular characterization of two Serine Proteinase Inhibitor genes in the surf clam *Mesodesma donacium* exposed to *Vibrio anguillarum*. **Maldonado-Aguayo, W.**[1], Chávez-Mardones, J.[1], Núñez-Acuña, G.[1], Valenzuela-Muñoz, V.[1], Gallardo-Escárate, C.[1]. Laboratorio de Biotecnología y Genómica Acuícola, Departamento de Oceanografía, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción. P.O. Box 160-C. Concepción, Chile.[1]. Sponsored by Uribe P, E.A.

The aim of this study was to characterize the full mRNA sequence of serine protease inhibitors (SPI) in the surf clam *Mesodesma donacium* and evaluate its association with innate immunity response. Herein, two *SPI* were identified from EST-data set generated by 454-pyrosequencing and designated as *MdIsp2* y MdIsp1. Specific primers were designed to obtain the ORF regions, and also Rapid Amplification of cDNA End (RACE) to reveals the 5? and 3? UTRs was performed. The sequences showed a characteristic Kazal domain with six conserved cysteines, responsible for the formation of disulfide bridges (C1x (3) C2X (7) PVC3X (3) GX2YXNXC4X (6) C5X (12) C). Real time PCR analysis shown that MdIsp1 is over-expressed in the mantle tissue over the foot, gill or muscle. MdIsp2 is expressed mainly in foot tissue. Phylogenetic analyzes show that these sequences form a clade closely related to bivalves. Further, exposition trails to *Vibrio anguillarum* revealed that the relative mRNA expression levels of MdIsp1 and MdIsp2 were up regulated at 2 and 8 h. These results suggest that MdIsp2 and MdIsp1 can be involved in antibacterial immune response in mollusk bivalves. Funding FONDEF 091065.

147.Ubiquitin-conjugating enzyme E2-like gene associated to pathogen response in *Concholepas concholepas*: SNP identification and transcription expression. **Aguilar-Espinoza, A.**[1], Núñez-Acuña, G.[1], Chávez-Mardones, J.[1], Gallardo-Escárate, C.[1]. Laboratorio de Biotecnología y Genómica Acuícola, Departamento de Oceanografía, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción. P.O. Box 160-C. Concepción, Chile.[1]. Sponsored by Uribe P, E.A.

Ubiquitin-conjugated E2 enzyme (UBE2) is one of the main components of the proteasome degradation cascade. Previous studies have shown an increase of expression levels in individuals challenged to some pathogen organism such as virus and bacteria. The aim of this study was to characterize the immune response of *UBE2* gene in the gastropod *Concholepas concholepas* through expression analysis and single nucleotide polymorphisms (SNP) discovery. Hence, UBE2 was identified from a cDNA library by 454 pyrosequencing, while SNP identification and validation were performed using De novo assembly and high resolution melting analysis. Challenge trials with *Vibrio anguillarum* was carried out to evaluate the relative transcript abundance of UBE2 gene from two to thirty-three hours post-treatment. The results showed a partial UBE2 sequence of 889 base pair (bp) with a partial coding region of 291 bp. A SNP variation (A/C) was observed at the 546th position. Individuals challenged by V. anguillarum showed an overexpression of the UBE2 gene, the expression being significantly higher in homozygous individuals (AA) than (CC) or heterozygous individuals (A/C). This study contributes useful information relating to the UBE2 gene and its association with innate immune response in marine invertebrates. Funding: FONDEF D09I1065.

148.Functional studies of the PcAce1 transcriptional factor from the fungus *Phanerochaete chrysosporium* by copper. **Silva, M.**[1], Essus, K.[1], Riffo, M.[1], Bull, P.[1]. Pontificia Universidad Católica de Chile[1].

Two transcription factors, Ace1 and Mac1, from *Saccharomyces cerevisiae* have been shown to participate in copper homeostasis. Ace1 is activated at high copper concentration (above 10 uM), whereas Mac1 is activated at low concentration (below 1 uM). This regulatory function depends on the presence of particular Cys motifs in their amino acid sequence. These motifs contain arrangements of the type Cys-X-Cys/His and Cys-X2-His. Transcription factor PcACE1 from *Phanerochaete chrysosporium*, a white rot fungus, contains Cys motifs similar to Ace1 in the amino terminus and to Mac1 in the carboxy terminus. Another feature present in PcAce1, but absent in both factors is a Ser-rich domain localized to the carboxy terminus. In order to discover the importance of these domains in PcAce1, site directed mutagenesis was performed to obtain fragments with different length, which vary at the carboxy terminus. Each mutant was evaluated for growth and transcriptional activity at different high copper concentration in recombinant *Saccharomyces cerevisiae ace*1-1, DTY59 and DTY23 respectively. The results show that the first four Cys motifs are critical for survival at high copper concentrations, and also are essential for the transcription factor to be an active protein that induces target genes. Furthermore, the region between the fifth Cys motif and Ser-rich domain inhibits both functions. **Acknowledgements:** This work was supported by Proyecto Puente VRI (Vicerrectoria de Investigación PUC) 2761-043.

149.Expression of the N-terminal half *Neisseria meningitidis* TbpB gene in *Salmonella* oral vaccines strains: immunogenicity in BALB/c mice compared to the entire TbpB gene. **Touma, J.**[1], Bruce, E.[1], Ávila, A.[1], Hernández, C.[1], Venegas, A.[1]. Laboratory of Microbial Pathogenesis and Vaccine Biotechnology. Department of Molecular Genetics and Microbiology, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Portugal 49, Santiago[1].

Neisseria meningitidis B is a major causative agent of meningitis, particularly in Chile. The disease affects mostly infants, leaving sequels and causing number of deaths. The lack of effective polysaccharide vaccines against serogroup B prompted us to search for new protein antigens as an alternative. Our previous studies demonstrated that TbpB (transferrin binding protein B) induced bactericidal antibodies in orally immunized mice. Given the large size of TbpB (86 kDa) it was quite difficult to obtain appropriate levels of TbpB expression in vaccine strains. To optimize this, a gene fragment encoding N-terminal TbpB half, containing most of residues involved in human transferrin binding was selected for further studies. These were focused on the comparison of two *Salmonella* vaccine strains used for oral administration and antigen delivery to the mice intestinal mucose. In addition, we used two tbpB genes encoding serotypes with 87.3% amino acid identity as source of short and full-length antigen versions. These segments were cloned in plasmids and transferred to *S. typhimurium* X4550 and *S. enteritidis* delta-pncB strains, the last one developed by us. Adequate expression of entire TbpB and N-terminal halves in *Salmonella* cultures was demonstrated by Western blotting. These four constructions in both strains were used to immunize BALB/c mice and evaluate IgG sera response against TbpB. Data showed that N-terminal halves raised higher response than full-length antigens. Moreover, *S. typhimurium* X4550 induced higher IgG levels than *S. enteritidis* delta-pncB strain, being a better vaccine candidate.

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