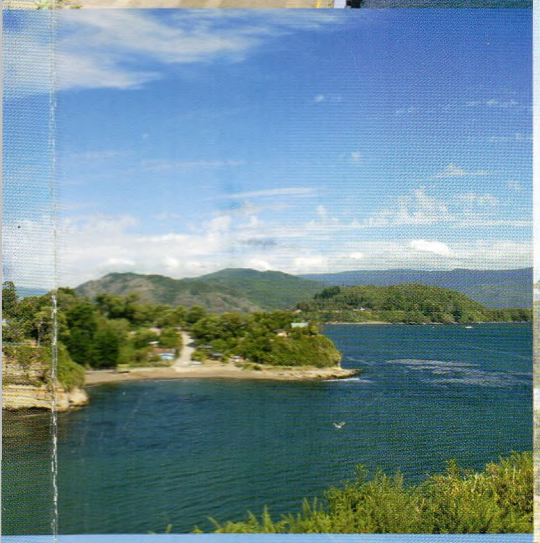
XXXIV REUNIÓN ANUAL SOCIEDAD DE BIOQUÍMICA Y BIOLOGÍA MOLECULAR DE CHILE



Hotel Dreams Pedro de Valdivia, Valdivia • 27 - 30 septiembre 2011

XXXIV REUNIÓN ANUAL SOCIEDAD DE BIOQUÍMICA Y BIOLOGÍA MOLECULAR DE CHILE

27 - 30 de septiembre del 2011
Valdivia - Chile

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PROGRAMA

Tuesday, September 27th

11:00- 13:00 Registration
13:00- 14:30 Lunch

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

STRUCTURE AND FUNCTION OF MACROMOLECULES
Salón Río Cruces

Chair: Óscar León
Co-chair: Rodolfo Amthauer

15:00 **Standardized comparison of structural alignments of catalytic domains in DNA polymerases from different methods. Slater, A.W.**[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, Alameda 340, Santiago, Chile.[2].

15:15 **Following cold denaturation of phosphofructokinase-2 by amide hydrogen/deuterium (H/D) exchange mass spectrometry. Ramírez, C.**[1], Baez, M.[1], Wilson, C.A.M.[1], Babul, J.[1], Komives, E.[2], Guixé, V.[1]. Department of Biology, Faculty of Sciences, University of Chile, Santiago, Chile.[1], Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla CA 92093-0359, USA[2].

15:30 **Docking and in-vitro study of human lipoxygenases inhibitors. Mascayano, C.**[1], Espinosa, V.[2], Sepulveda-Boza, S.[2], Holman, T.[3]. Departamento de Ciencias del Ambiente, Facultad de Química y Biología,Universidad de Santiago de Chile[1], Escuela de Medicina, Facultad de Ciencias Médicas, Universidad de Santiago[2], Department of Chemistry and Biochemistry, University of California, Santa Cruz[3]. Sponsored by F. Gonzalez Nilo.

15:45 **Widen minor groove is a common feature in damaged DNA and protein-damaged-DNA complexes. Cifuentes, 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, Alameda 340, Santiago, Chile[2].

16:00 **Quenching of the intrinsic fluorescence of *Chlorobium tepidum* Ferritin by Iron. The role of the Ferroxidase Center in the mineralization process. Yévenes, A.**[1], Espinoza, R.[2], Márquez, V.[3], Sandoval, C.[1], González-Nilo, F.[1], Lopez-Castro, J.[4], Domínguez-Vera, J.M.[5], Watt, R.K.[6]. Universidad de Talca[1], Universidad de Santiago[2], Fraunhofer Chile Research[3], Universidad de Cadiz[4], Universidad de Granada[5], Brigham Young University[6].

16:15 **The 5`untranslated region of the HIV-2 mRNA harbors an internal ribosome entry site element. Letelier, A.**[1], López-Lastra, M.[1]. Laboratorio de Virología Molecular, Centro de Investigaciones Médicas, Facultad de Medicina, Pontificia Universidad Católica de Chile[1].

16:15 **Development of antibodies against iNOS of fish: A molecular marker of immune response in gill of rainbow trout. Valenzuela, C.**[1], Guzmán, F.[2], Rojas, V.[1], Mercado, L.[1,2]. Grupo de Marcadores Inmunológicos en Organismos Acuáticos. Laboratorio de Genética e Inmunología Molecular (GIM), Instituto de Biología[1], Núcleo Biotecnológico Curauma (NBC). Pontificia Universidad Católica de Valparaíso, Chile[2].

16:45 **Iodide (I^-) induce a rapid inhibition of Na^+/I^- symporter (NIS) through reactive oxygen species. Arriagada, A.**[1], Becerra, Á.[2], Miranda, C.[1], Plaza, A.[1], Simon, F.[2], Bueno, S.[3], Kalergis, A.[3], Carrasco, N.[4], Riedel, C.[1]. Laboratorio de Biología Celular y Farmacología. Departamento de Ciencias Biológicas. Universidad Andrés Bello[1], Laboratorio de Fisiopatología Celular y Molecular, Departamento de Ciencias Biológicas, Univerisdad Andrés Bello.[2], Millennium Institute on Immunology and Immunotherapy. Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas. Pontificia Universidad Católica de Chile.[3], Department of Molecular Pharmacology, Albert Einstein College of Medicine, USA[4].

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

MOLECULAR BIOLOGY OF THE CELL I
Salón Río Calle Calle

Chair: Andrew Quest
Co-chair: Juan Olate

15:00 **Evaluation of antitumor therapy in a murine model of bladder cancer. Rivas, A.**[1,2,3], Borgna, V.[1,2,3], Landerer, E.[1,3], Avila, M.[1,3], Burzio, V.[1,2,3], Villegas, J.[1,2,3]. Andes Biotechnologies S.A.[1], Fundación ciencia para la vida[2], Universidad Andres Bello[3].

15:15 **Draft genome assembly of *Penicillium purpurogenum* using next-generation sequencing techniques and identification of possible lignocellulolytic enzyme genes. Mardones, W.**[1], Eyzaguirre, J.[1]. Universidad Andrés Bello[1].

15:30 **Toxin-antitoxin systems in a mobile genetic element from *Acidithiobacillus ferrooxidans*. Bustamante, P.**[1], Orellana, O.[1]. Programa de Biología Celular y Molecular, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile[1]

15:45 **Human metapneumovirus impairs the capacity of dendritic cells to prime antigen-specific naïve T cells. Céspedes, P.F.**[1,2], González , P.A.[1,2], Kalergis, A.M.[1,3]. Millennium Institute on Immunology and Immunotherapy.Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile[1], These authors contributed equally[2], Departamento de Reumatología. Facultad de Medicina. Pontificia Universidad Católica de Chile[3].

16:00 **Caveolin-1 phosphorylation on tyrosine-14 sensitizes b-pancreatic Min-6 cells to palmitate-induced apoptosis. Núñez, S.**[1], Mears, D.[2], Leyton, L.[1], Quest, A.[1]. Centro Fondap de Estudios Moleculares de la Célula (CEMC), Facultad de Medicina, Universidad de Chile[1], USU School of Medicine, Bethesda, Maryland, USA[2].

16:15 **Proinflammatory cytokines as potential immunological markers in rainbow trout (*Oncorhynchuss mykiss*) larvae. Santana, P.**[1], Guzmán, F.[2], Mercado, L.[1,2]. Grupo de Marcadores Inmunológicos en Organismos Acuáticos. Laboratorio de Genética e Inmunología Molecular (GIM), Instituto de Biología[1], Núcleo Biotecnológico Curauma (NBC). Pontificia Universidad Católica de Valparaíso. Chile[2].

16:30 **IgG prevents subversion of dendritic cell phagocytosis by virulent *Salmonella* and restores antigen presentation to T cells. Riquelme, S.**[1], Bueno, S.[1], Kalergis, A.[1,2]. Millennium Institute of Immunology and Immunotherapy. Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas. Pontificia Universidad Católica de Chile[1], Departamento de Reumatología, Facultad de Medicina. Pontificia Universidad Católica de Chile[2].

16:45 **Development of polyclonal antibody anti-Vtg and an enzyme-linked immunosorbent assay ELISA for vitellogenin measurement in Paralichthys adspersus used in endocrine disruptor screening. Leonardi, M.**[1,2], Bustos, P.[3], Puchi, M.[4], Romo, X.[1], Morin, V.[4]. Departamento de Ciencias Biologicas, Facultad de Ciencias Biologicas, Universidad Andres Bello[1], Programa de Investigacion Marina de Excelencia (PIMEX-Nueva Aldea), Universidad de Concepcion[2], Departamento de Bioquimica Clinica e Inmunologia, Facultad de Farmacia, Universidad de Concepcion[3], Departamento de Bioquímica y Biologia Molecular, Facultad de Ciencias Biologicas, Universidad de Concepcion[4].

17:30-19:00 SYMPOSIUM: RNA: structure, function and modelling
Salón Río Tornagaleones

Chair: Marcelo Lopez-Lastra

17:30 **Development of bioinformatics tools to assist the inference of sequence-structure-function relationships in small RNAs.** Norambuena, T.[1,2], **Melo, F.**[1,2]. Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Alameda 340, Santiago, Chile[1], Molecular Bioinformatics Laboratory, Millennium Institute on Immunology and Immunotherapy, Santiago, Chile[2]

18:00 **Loading the ribosome on the mRNA, the intriguing case of HIV.** Chamond, N.[1], Deforges, J.[1], Brossard, A.[1], Ulryck, N.[1], **Sargueil, B.**[1]. UMR 8015, Laboratoire de cristallographie et RMN Biologique, Université Paris Descartes, 4 avenue de l'Observatoire, 75270 Paris Cedex 06. Francia[1].

18:30 **In vitro studies of the molecular mechanisms involved in miRNA repression.** Ricci, E.P.[1,2,3], Limousin, T.[1,2,3], Soto Rifo, R.[1,2,3], Decimo, D.[1,2,3], **Ohlmann, T.**[1,2,3]. Ecole Normale Supérieure de Lyon, Unité de Virologie Humaine, IFR 128, Lyon, F-69364 Franch[1], Inserm, U758, Lyon, F-69364 France[2], Université de Lyon[3].

19:15-20:15 OPENING PLENARY
Salón Río Tornagaleones

19:15 **Grabbing the Cat by the Tail: Discrete Steps and Inter-Subunit Coordination by a DNA Packaging Ring-ATPase. Bustamante, C.**[1]. University of California, Berkeley[1].

20:30 Cocktail and Dinner

22:00-24:00 POSTER SESSION I (Odd numbers, see poster numbering on page 16)
Salón Salón Río Valdivia

Chair: Amalia Sapag

Wednesday, September 28th

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

PROTEINS STRUCTURE AND FUCTION
Salón Río Cruces

Chair: Cecilia Rojas
Co-chair: Ana Preller

09:00 **Nucleotide selectivity mechanism of the ADP-dependent glucokinase from *Thermococcus litoralis*. Merino, F.**[1], Guixé, V.[1]. Laboratorio de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Chile[1]

09:15 **The role of Heme oxygenase 1 in Systemic Lupus Erithematosus. Mackern, J.P.**[1], Herrada, A.[2], Carreño, L.J.[3], Gómez, R.S.[2], Anegón, I.[4], Jacobelli, S.H.[5], Llanos, C.[5], Kalergis, A.M.[2,5,6]. Millennium Institute on Immunology and Immunotherapy. Departamento de Genética Molecular. Facultad de Ciencias Biológicas. Pontificia Universidad Católica de Chile, Santiago, Chile[1], Millennium Institute on Immunology and Immunotherapy. Departamento de Genética Molecular. Facultad de Ciencias Biológicas. Pontificia Universidad Católica de Chile, Santiago, Chile[2], Millennium Institute on Immunology and Immunotherapy. Departamento de Genética Molecular. Facultad de Ciencias Biológicas. Pontificia Universidad Católica de Chile, Santiago, Chile[3], INSERM UMR 643, CHU de Nantes. France[4], Departamento de Reumatología, Facultad de Medicina. Pontificia Universidad Católica de Chile, Santiago, Chile[5 6]

09:30 **Independence between thermal and mechanical stability in a hyperthermophilic enzyme. Single molecule studies reveal the modulation of the mechanical stability triggered by ligands. Rivas-Pardo, J.A.**[1], Alegre-Cebollada, J.[2], Ramírez, C.[1], Fernández, J.M.[2], Guixé, V.[1]. Facultad de Ciencias, Universidad de Chile, Santiago, Chile[1], Department of Biological Sciences, Columbia University, New York, USA[2]

09:45 **Expression and biochemical analysis of AtSDL, a putative sorbitol dehydrogenase in *Arabidopsis thaliana*. Aguayo, F.**[1], Ampuero, D.[1], Parada, R.[1], Mandujano, P.[1], Handford, M.[1]. Laboratorio de Biología Molecular Vegetal, Departamento de Biología, Facultad de Ciencias, Universidad de Chile[1].

10:00 **Interactions defining the strong specificity for NADP in *Escherichia coli* glucose-6-P dehydrogenase. Cabrera, R.**[1], Fuentealba, M.[2], Muñoz, R.[3] Departamento de Biología, Facultad de Ciencias, Universidad de Chile[1], Universidad de Chile[2], Universidad Andres Bello[3].

10:15 **Structural-functional determinants of the intracellular ascorbic acid binding domain in SVCT1. Haensgen, H.**[1], Salas-Burgos, A.[1], Sepulveda, M.A.[1], Rivas, C.[1], Vera, J.C.[1]. Departamento de Fisiopatología, Facultad de Ciencias Biológicas, Universidad de Concepción. Concepción, Chile[1]

10:30 **Effect of citrate on the activity of mammalian Fructose 1,6-bisphosphatase. Asenjo , J.L.**[1], Díaz, A.[2], Maureira, M.A.[1], Schott , S.[1], Yáñez, A.J.[1], Guinovart, J.J.[2], Slebe, J.C.[1]. Instituto de Bioquímica y Microbiología, Universidad Austral de Chile, Valdivia[1], Institut de Recerca Biomédica, Barcelona, Spain[2]

10:45 **Characterization of *Daucus carota* lycopene b-cyclase gene (*lcyb1*) by over-expression in plant models. Moreno, J.C.**[1], Stange, C.[1]. Facultad de Ciencias, Universidad de Chile[1]

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

GENE EXPRESSION I
Salón Río Calle Calle

Chair: Rosalba Lagos
Co-chair: Claudia Stange

09:00 **Identification and functional characterization of *ERF115* gene coding for a transcription factor involved in tolerance to high salinity stress in *Arabidopsis thaliana.* León, L.**[1], Casaubon, M.J.[1], Villaroel, E.[1], Holuigue, L.[1]. Plant Molecular Biology Laboratory and Millennium Nucleus for Plant Functional Genomics, Department of Molecular Genetics and Microbiology, Pontifical Catholic University of Chile[1].

09:15 **Annotation of putative AraC/XylS-family transcription factors with unknown function. Schüller, A.**[1,2], 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[2].

09:30 **Optimizing homologous recombination in slow growing mycobacteria. Gonzalez, P.**[1,2,3,4,5], Jain, P.[4,5], Hsu, T.[4,5], Jacobs Jr., W.[4,5]. Millennium Institute on Immunology and Immunotherapy. Departamento de Genética[1], Molecular y Microbiología, Facultad de Ciencias Biológicas. Pontificia Universidad Católica de[2], Chile, Santiago, Chile.[3], Howard Hughes Medical Institute and Department of Microbiology and[4], Immunology, Albert Einstein College of Medicine, Bronx, NY, USA[5]. Sponsored by A. Kalergis.

09:45 **Full-atom structure-based prediction of transcription factor binding sites. 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].

10:00 **The unfolded protein response has a circadian component in *Neurospora crassa.* Goity, A.**[1], Montenegro-Montero, A.[1], Larrondo, L.F.[1]. Depto. Genética Molecular y Microbiología, Facultad Ciencias Biológicas, Pontificia Universidad Católica de Chile[1]

10:15 **Transcriptional regulation of an embryogenic-specific iron-sulfur protein isoform of *Arabidopsis thaliana* mitochondrial complex II. Restovic, F.**[1], Roschzttardtz, H.[1], Vásquez, M.[1], Gómez, M.I.[1], Vicente-Carbajosa, J.[2], Jordana, X.[1]. Pontificia Universidad Católica de Chile[1], Universidad Politécnica de Madrid[2].

10:30 **Transcriptional regulation of trafficking-genes in *Arabidopsis thaliana*. Pizarro, L.**[1,2], Vergara, A.[1], Gutierrez, R.[3], Rojas-Pierce, M.[4], Norambuena, L.[1,2]. Plant Molecular Biology Laboratory. Faculty of Science. University of Chile[1], Plant Cell Biotechnology Millennium Nucleus.[2], Plant System Biology Lab. Molecular Genetic and Microbiology Department. Faculty of Biological Science. Pontifical Catholic University of Chile[3], Department of Plant Biology. North Carolina State University. Raleigh, NC[4].

10:45 **The transcription factor CREB binds to the proximal region of the human RIC-8B promoter. Maureira Moya, A.**[1], María Victoria, H.[1], José Leonardo, G.[2], Juan, O.[1]. Laboratorio de Genética Molecular, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción[1], Laboratorio de Regulación Transcripcional, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción[2].

11:30-13:00 SYMPOSIUM: STRUCTURAL BASIS OF COMMUNICATION IN PROTEINS
Sala Río Tornagaleones

Chair: Victoria Guixé

11:30 **The regulatory mechanism of phosphofructokinase-2 from *E. coli* by ATP: allosteric transitions characterized by X-ray crystallographic, biochemical and bioinformatics methods. Baez, M.**[1]. Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago[1].

12:00 **Functional consequences of intrinsic disorder in the NFκB-IκB interaction. Komives, E.**[1]. Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla CA 92093-0359, USA[1]

12:30 **Communication in phycobilisomes from *Gracilaria chilensis*. Bunster, M.**[1] Depto. Bioquimica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción[1]

13:00 Lunch

14:30-16:30 ORAL SESSION 5
GENE EXPRESSION II
Salón Río Cruces

Chair: Luis Burzio
Co-chair: Gino Corsini (Claudia Stange)

14:30 **Systems approaches map regulatory networks downstream of the auxin receptor *AFB3* in the nitrate response of *Arabidopsis thaliana*. Vidal, E.A.**[1], Moyano, T.C.[1], Riveras, E.[1], Gutiérrez, R.A.[1]. FONDAP Center for Genome Regulation, Millennium Nucleus Center for Plant Functional Genomics, Departamento de Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile[1]

14:45 **Identification of two proteins involved in the control of rhythmic gene expression in *Neurospora crassa*. Olivares-Yañez, C.**[1], Muñoz, F.[1], Larrondo, L.F.[1]. Depto. Genética Molecular y Microbiología, Facultad Ciencias Biológicas. Pontificia Universidad Católica de Chile[1].

15:00 **Participation of light and the circadian clock in the pathogenicity of *Botrytis cinerea*. Hevia, M.**[1], Canessa, P.[1], Larrondo, L.F.[1]. Depto. Genética Molecular y Microbiología, Facultad Ciencias Biológicas, Pontificia Universidad Católica de Chile[1]

15:15 **Herp stimulates cytoprotection against oxidative stress by regulating intracellular calcium. Paredes, F.**[1], Quiroga, C.[1], Jaimovich, E.[1,2], Lavandero, S.[1,2]. FONDAP CEMC, Facultad de Ciencias Química y Farmacéuticas/Facultad de Medicina, Universidad de Chile[1], ICBM, Facultad de Medicina, Universidad de Chile[2]

15:30 **Expression of a Mg^{2+}-dependent HIV-1 RNase H in a single polypeptide for drug screening. Vargas, D.A.**[1], Castillo, A.E.[1], Roth, M.J.[2], Leon, O.[1]. Programa de Virología, ICBM, Facultad de Medicina. Universidad de Chile[1], UMDNJ-RWJMS, Biochemistry, Piscataway, NJ[2]

15:45 **The Andes Hantavirus NSs protein is expressed from the viral small mRNA by a leaky scanning mechanism. Solis, L.**[1], Vera, J.[1], Pino, K.[1], López-Lastra, M.[1]. Laboratorio de Virologia Molecular, Centro de investigaciones Medicas, Facultad de Medicina, Pontificia Universidad Catolica[1]

16:00 **Regulation of non-coding mitochondrial RNA by a High Risk Human Papillomavirus (HPV) protein. Campos, A.**[1,2,3], Varas, M.[1,2,3], Burzio E., L.[1,2,3], Villota, C.[1,2,3]. Andes Biotechnologies S.A[1], Fundación Ciencia para la Vida[2], Facultad de Ciencias, UNAB[3]. Sponsored by J. Villegas.

16:15 **Genetic association between host IL28B genotype and extrahepatic HCV infection. Angulo, J.**[1,2], Pino, K.[1], Biel, F.[3], Soza, A.[3], López-Lastra, M.[1]. Laboratorio de Virología Molecular, Instituto Milenio de Inmunología e Inmunoterapia (IMII), Centro de Investigaciones Médicas, Facultad de Medicina, Pontificia Universidad Católica de Chile[1], Programa de Doctorado en Microbiología (USACH)[2], Departamento de Gastroenterología, Facultad de Medicina, Pontificia Universidad Católica de Chile[3]

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

MOLECULAR BIOLOGY OF THE CELL II
Salón Río Calle Calle

Chair: Patricio Arce-Johnson
Co-chair: Alejandra Moya

14:30 **HsUGTrel1 is a human UDP-glucose transporter with three splicing variants that are upregulated by the unfolded protein response.**Donoso, M.[1], Moreno, I.[1], Temple, H.[1], Moreno, A.[1], **Orellana, A.**[1]. FONDAP Centro de Regulación del Genoma, Núcleo Milenio en Biotecnología Celular Vegetal,Centro de Biotecnología Vegetal, Facultad de Ciencias Biológicas, Universidad Andrés Bello[1].

14:45 **Knock-down of the Sense non-coding mitochondrial RNA (SncmtRNA) in normal cells line induces modulation in the cell cycle. Vidaurre, S.**[1], Oliveira-Cruz, L.[2], Fitzpatrick, C.[1], Villegas, J.[1], Burzio , V.[1], Burzio , L.[1]. Andes Biotechnologies, Fundación ciencia para la vida, UNAB[1], Andes Biotechnologies, Fundación ciencia para la vida[2].

15:00 **Molecular effect of lithium on Sertoli cells. Maldonado, R.**[1], Villarroel-Espíndola, F.[1], Torres, C.[1], Cereceda, K.[1], Van der Stelt, K.[1], Lopez, C.[1], Salazar, E.[1], Covarrubias, A.[1], Angulo, C.[1], Castro, M.[1], Slebe, J.C.[1], Concha, I.I.[1]. Instituto de Bioquímica y Microbiología, Universidad Austral de Chile[1].

15:15 **AtDFG10-2 plays a role in N-glycosylation, endomembrane trafficking and development in *Arabidopsis thaliana*. Rubilar, C.**[1,2], Norambuena, L.[1,2]. Laboratorio de Biología Molecular Vegetal, Facultad de Ciencias, Universidad de Chile[1], Núcleo Milenio en Biotecnología Celular Vegetal[2].

15:30 **Differential regulation of endoplasmic reticulum-mitochondria coupling by mTOR inhibition and tunicamycin-induced endoplasmic reticulum stress. Bravo, R.**[1], Parra, V.[1], Rodríguez, A.E.[1], Quiroga, C.[1], Paredes, F.[1], Quest, A.F.[1,2], Lavandero, S.[1,2]. FONDAP CEMC, Facultad de Ciencias Químicas y Farmacéuticas/Facultad de Medicina, Universidad de Chile[1], ICBM, Facultad de Medicina, Universidad de Chile[2].

15:45 **The PIP5K1 and 2 enzymes are required for the normal reproductive development in *Arabidopsis thaliana*. Ugalde, J.M.**[1], Tejos, R.[2], Friml, J.[2], León, G.[1]. Laboratorio de Reproducción y Desarrollo de Plantas, Centro de Biotecnología Vegetal, Universidad Andrés Bello[1], Department of Plant System Biology, VIB Research Institute and Gent Universitiet, Belgium[2].

16:00 **A novel mechanism for mTOR-dependent mitochondrial dynamics regulation. Verdejo, H.**[1,2], Parra, V.[3], Kuzmicic, J.[3], Lavandero, S.[3]. Division de Enfermedades Cardiovasculares Facultad de Medicina Pontificia Universidad Catolica de Chile[1], Programa Doctorado Ciencias Medicas Facultad de Medicina Pontificia Universidad Catolica de Chile[2], Centro FONDAP Estudios Moleculares de la Célula Facultad de Ciencias Químicas y Farmacéuticas/Facultad de Medicina Universidad de Chile[3].

16:15 **LLP, a plasma membrane lectin induced by salicylic acid and involved in the defense response against *Pseudomonas syringae* in *Arabidopsis thaliana*. Armijo, G.**[1], Seguel, A.[1], García, C.[1], Salinas, P.[1], Leiva , D.[1], Holuigue, L.[1]. Departamento de Genética Molecular y Microbiología, Facultad Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago de Chile[1].

17:00-19:00 **NEW MEMBERS SESSION I**

Salón Río Tornagaleones

Chair: Juan Carlos Slebe

Co-chair: María Antonieta Valenzuela

17:00 **Sorting of the Alzheimer's disease amyloid precursor protein mediated by the AP-4 complex. Mardones, G.**[1,2], Burgos, P.[1,2], Rojas, A.[3], da Silva, L.[2], Prabhu, Y.[2], Hurley, J.[3], Bonifacino, J.[2]. Instituto de Fisiología, Facultad de Medicina, Universidad Austral de Chile, Valdivia[1], Cell Biology and Metabolism Program, NICHD[2], Laboratory of Molecular Biology, NIDDK, NIH, Bethesda, MD, USA[3].

17:30 **Characterization of the binary complex [SdiA/ acyl-HSL] in the transcription of the *ftsQAZ* operon. There is a correlation between quorum sensing and cell division? Gallardo, M.J.**[1,2], Guiliani, N.[1], Monasterio, O.[1], Prevelige, P.[3], Bustamante, C.[4]. Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile[1], CEFOP, Facultad de Física y Matemáticas, Universidad de Concepción, Concepción, Chile[2], Department of Microbiology, University of Alabama at Birmingham, Birmingham, USA[3], University of California, Berkeley, California, USA., Howard Hughes Medical Institute, Berkeley, California, USA[4].

18:00 **Insulin signalling increases mitochondrial metabolism by promoting mitochondrial fusion. Parra, V.**[1], Verdejo, H.[1,2], Troncoso, R.[1], Kuzmicic, J.[1], del Campo, A.[1], Pennanen, C.[1], Lopez-Crisosto, C.[1], Chiong, M.[1], Zorzano, A.[3], Lavandero, S.[1]. FONDAP Center for Molecular Studies of the Cell, Facultad de Ciencias Químicas y Farmacéuticas / Facultad de Medicina, Universidad de Chile, Santiago 8380492, Chile[1], División de Enfermedades Cardiovasculares, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile[2], Institute for Research in Biomedicine (IRB Barcelona), Baldiri i Reixac, 10, 08028 Barcelona, Spain[3].

18:30 **Activity of the human immunodeficiency virus type 1 cell cycle-dependent internal ribosomal entry site is modulated by IRES trans-acting factors. Vallejos, M.**[1], Deforges, J.[2], Plank, T.M.[3], Letelier, A.[1], Ramdohr, P.[1], Abraham, C.G.[4], Valiente-Echeverría, F.[1], Kieft, J.S.[5], Sargueil, B.[2], Lopez-Lastra, M.[1]. Laboratorio de Virología Molecular, Instituto Milenio de Inmunología e Inmunoterapia, Centro de Investigaciones Médicas, Facultad de Medicina, Pontificia Universidad Católica de Chile, Marcoleta 391, Santiago, Chile[1], CNRS UMR 8015, Laboratoire de cristallographie et RMN Biologique, Universite Paris Descartes, 4 avenue de lObservatoire, 75270 Paris Cedex 06, France[2], Department of Biochemistry and Molecular Genetics, University of Colorado Denver School of Medicine, Aurora, CO, 80045, USA[3], Department of Microbiology, University of Colorado Denver School of Medicine, Aurora, CO, 80045, USA[4], Department of Biochemistry and Molecular Genetics and Howard Hughes Medical Institute, University of Colorado Denver School of Medicine, Aurora, CO, 80045, USA[5].

19:15-20:30 **PABMB CONFERENCE**

Salón Tornagaleones

19:15 **Light signalling networks in the control of plant development. Casal, J.J.**[1,2].
Fundación Instituto Leloir[1], Universidad de Buenos Aires, Argentina[2].

20:30 Dinner

22:00-24:00 **POSTER SESSION II (Even numbers, see poster numbering on page 16)**
Salón Río Valdivia

Chair: Soraya Gutiérrez

Thursday, September 29th

09:00-11:00 **SYMPOSIUM: MOLECULAR GENETICS ON MICROBES AND THE IMMUNE RESPONSE**
Salón Río Tornagaleones

Chair: Alexis Kalergis

09:00 **B cells in Trypanosoma cruzi infection: role as antibody-secreting cells and as cytokine producers. Gruppi, A.**[1]. Universidad Nacional de Córdoba, Argentina[1].

09:30 **Immunity to flagellated bacteria. McSorley, S.**[1]. University of California Davis, Center for Comparative Medicine[1].

10:00 **Impact of virulence genes acquired by lateral gene transfer in the immune response against Salmonella. Bueno, S.**[1,2]. Millennium Institute on Immunology and Immunotherapy[1], Facultad de Ciencias Biológicas. P. Universidad Católica de Chile[2].

10:30 **Coupling transcription regulation with DNA replication by the DnaA protein. Sclavi, B.**[1]. CNRS, ENS Cachan, France[1].

11:30-13:00 **OSVALDO CORI CONFERENCE**

Salón Tornagaleones

11:30 **BioHydrogen production by fermentation in an artificial bacterial ecosystem. Interbacterial species communication by physical contact?** Benomar, S.[1], Leroy, G.[1], Trably, E.[2], Giudici-Orticoni, M.[1], **Cárdenas, M.**[1]. Laboratoire de Bioénergétique et Ingénierie des Protéines, CNRS, 31 chemin Joseph Aiguier 13402 Marseille cedex 20 - France[1], Laboratoire de Biotechnologie de l'Environnement, INRA, Avenue des Etangs 11100 Narbonne, France [2].

13:00 Lunch

14:30-16:00 **NEW MEMBERS SESSION II**

Salón Río Tornagaleones

Chair: José Martinez
Co-chair: Ariel Castro

14:30 **Array CGH genomic profile of hereditary breast cancer tumors. Identification of tumor suppressor genes in deleted regions, determination of promoter hypermethylation and their protein expression in tumor biopsies. Álvarez, C.**[1], Tapia, T.[1], Cornejo, V.[2], Aravena, A.[3], Fernández, W.[2], Camus, M.[4], Alvarez, M.[5], Maass, A.[6], Carvallo, P.[1]. Depto de Biología Celular y Molecular, Fac de Ciencias Biológicas, P. Universidad Católica de Chile, Santiago[1], Unidad de Anatomía Patológica, Hospital San Borja Arriarán, Santiago[2], Centro de Modelamiento Matemático, Universidad de Chile[3], Centro de Cáncer, Fac de Medicina, P. Universidad Católica de Chile, Santiago[4], Clínica Las Condes, Santiago, Chile[5], Centro de Modelamiento Matemático, Universidad de Chile[6].

15:00 **Effects of Knock-Down of the Antisense Noncoding Mitochondrial RNAs on Cell Cycle and Viability of Normal and Tumor Cells. Burzio, V.A.**[1,2,3], Vidaurre, S.[1,2,3], Fitzpatrick, C.[1,2,3], Oliveira-Cruz, L.[1,2,3], Echenique, J.[1,2], Briones, M.[1,2,3], Villegas, J.[1,2,3,4], Burzio, L.O.[1,2,3,4]. Fundacion Ciencia para la Vida[1], Andes Biotechnologies SA[2], Universidad Andrés Bello[3], GrupoBios SA[4].

15:30 **Inhibition of Cyclin-Dependent Kinase 5 but Not of Glycogen Synthase Kinase 3-b prevents Neurite Retraction and Tau Hyperphosphorylation Caused by Secretable Products of Human T-Cell Leukemia Virus Type I-Infected Lymphocytes. Maldonado, H.**[1,2], Ramirez, E.[3], Utreras, E.[4], Pando, M.[1], Kettlum, A.[1], Chiong, M.[1], Kulkarn, A.[4], Collados, L.[1], Puente, J.[1], Cartier, L.[5], Valenzuela, M.[1]. Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas,Universidad de Chile[1], Laboratorio de Comunciaciones Celulares, Facultad de Medicina, Universidad de Chile[2], Programa de Virología, Departamento de Virología, Facultad de Medicina, Universidad de Chile[3], Functional Genomics Section, Laboratory of Cell and Developmental Biology, NIDCR, NIH[4], Departamento de Ciencias Neurológicas, Facultad de Medicina, Universidad de Chile[5].

16:00 **Distributed Structures Underlie Gating Differences between the Kin Channel KAT1 and the Kout Channel SKOR.** Riedelsberger, J.[1], Sharma, T.[1], **Gonzalez, W.**[2], Gajdanowicz, P.[1], Morales-Navarro, S.E.[2], Garcia-Mata, C.[3], Mueller-Roeber, B.[1], Gonzalez-Nilo, F.D.[2], Blatt, M.R.[3], Dreyer, I.[1] Universitat Potsdam, Germany[1], Universidad de Talca, Chile[2], University of Glasgow, UK[3].

17:30 **SEVERO OCHOA CONFERENCE**

18:30

Salón Tornagaleones

17:30 **A new function for CPEB: nuclear processing of pre-mRNAs.** Alessio, F.[1], **Méndez, R.**[1]. ICREA and Institute for Research in Biomedicine (IRB). Barcelona, Spain[1].

19:00 **SBBM MEMBERS MEETING**

20:30 Dinner

Friday, September 30th

09:45-10:35 **PABMB SYMPOSIUM:**
INNOVATIVE STRATAGIES FOR BIOCHEMISTRY LEARNING
Salón Tornagaleones

Chair: Raúl Herrera

09:45 **Innovative strategies for teaching biochemistry. Herrera R.**[1], Moya-Leon M.A.[1] Instituto Biologia Vegetal y Biotecnologia, Universidad de Talca[1]

09:55 **Experimental Biochemistry Simulation. Claude, A.**[1], von Chrismar Parejo, A.M.[1]. Instituto de Bioquímica y Microbiología, Universidad Austral de Chile[1].

10:15 **New strategies in the teaching of biochemistry at the University of Concepción. Martínez Oyanedel, J.**[1], Bruna, C.[1], Bunster, M.[1]. Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción[1].

10:35 **The Information Technologies and the Teaching of Biochemistry. Galembeck, E.**[1]. Depto. de Bioquímica - IB - UNICAMP, Campinas - SP - Brasil[1].

11:45-12:30 **Herman Niemeyer Medal Award**

12:30-13:00 **Best Oral, Poster and New Members Awards**

POSTER NUMBERING

1 **RNA aptamers: a new approach for the diagnosis and therapy of envenomation by *Loxosceles* spiders. Constenla-Muñoz, C.**[1], 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].

2 **Dopamine and spermatozoa; a relationship to be understood. Urra, J.**[1], Villarroel, F.[2], Lopez, C.[2], Rodríguez-Gil, J.E.[3], Ramírez, A.[4], Concha G, I.I.[2]. Instituto de Bioquímica y Microbiología, Instituto de Ciencia Animal, Universidad Austral de Chile[1], Instituto de Bioquímica y Microbiología, Universidad Austral de Chile[2], Unitat Reproducció Animal, Universitat Autonoma de Barcelona, España[3], Instituto de Ciencia Animal, Universidad Austral de Chile[4].

3 **Down expression of insulin receptor in human renal cortex of diabetic patients. Gatica, R.**[1,2,3,4], Kairath, P.[2], Caelles, C.[4], Slebe, J.C.[2], Yañez, A.[2]. Escuela de Graduados Facultad de Ciencias Veterinarias[1], Instituto de Bioquímica y Microbiología Universidad Austral de Chile[2], Universidad San Sebastián Sede Puerto Montt[3], Institute for Research in Biomedicine Barcelona España[4].

4 **Transcriptomic network analysis of *Arabidopsis thaliana* reveals specific gene regulation under cold, salt and UV-B stress conditions. Sagredo C, E.**[1,2], Espinoza, J.A.[2,3], Bizama, C.[2], Cabrera, G.[2], Gutiérrez Moraga, A.[2,3]. Carrera de Biotecnología, Universidad de La Frontera,Temuco, Chile[1], VentureL@b, Universidad Adolfo Ibáñez, Chile.[2], Programa de Doctorado en Ciencias Mención Biología Celular y Molecular Aplicada, Universidad de La Frontera,Temuco, Chile[3].

5 **Determination of the binding site of phosphatidylinositol-bisphosphate (PIP2) to transient receptor potential channels (TRP). Poblete, H.**[1], Oyarzun, I.[2], González-Nilo, D.[1,2], Latorre, R.[2]. Centro de Bioinformática y Simulación Molecular, Universidad de Talca, 2 Norte 685, Casilla 721, Talca, Chile[1], Centro Interdisciplinario de Neurociencias de Valparaíso, Facultad de Ciencias, Universidad de Valparaíso, 287 Gran Bretaña, Valparaíso 2360102, Chile[2].

6 **Structural characterization and substrate affinity of two different isoforms of alcohol acyltransferase from *Cucumis melo* involved in ester biosynthesis. Galaz, S.**[1], Morales-Quintana, L.[1], Moya-Leon, M.A.[1], Herrera, R.[1]. Instituto de Biología Vegetal y Biotecnología, Universidad de Talca[1].

7 **Deep-sequencing analysis of small RNAs in the *Xenopus tropicalis* gástrula. Almonacid, L.**[1,2], Faunes, F.[3], Lee-Liu, D.[3,4], 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], Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile[4].

8 **Dendritic nanoparticles (PAMAM) used as an optimal mechanism for transport and controlled drug delivery. Vergara-Jaque, A.**[1], Monsalve, L.[1], Sandoval, C.[1,2], González-Nilo, D.[1,2]. Center for Bioinformatics and Molecular Simulation, Universidad de Talca, 2 Norte 685, Casilla 721, Talca, Chile[1], Nanobiotechnology Division at University of Talca, Fraunhofer Chile Research Foundation - Center for Systems Biotechnology, FCR-CSB, Talca, Maule, Chile[2].

9 **Small molecules determination based in the Gβγ-GlyR interaction site. Cerda, F.**[1], San Martín, L.[1], Martínez, J.[2], Jiménez, V.[3], Guzmán, L.[1]. Departamento de Fisiología, Universidad de Concepción, Concepción, Chile[1], Departamento de Bioquímica y Biología Molecular, Universidad de Concepción, Concepción, Chile[2], Departamento de Química Orgánica, Universidad de Concepción, Concepción, Chile[3].

10 **Role of solvent channel in substrate selectivity of two different alcohol acyltransferase from climacteric fruit species. 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 **Structural characterization of PhpAAT1 enzyme and its importance in ester production in *Physalis peruviana*. Carrasco-Orellana, C.**[1], Morales-Quintana, L.[1], Zuñiga, R.[1], Moya-León, M.A.[1], Herrera, R.[1]. Instituto de Biología Vegetal y Biotecnología, Universidad de Talca[1].

12 **Dissecting functional domains on nucleotide sugar transporters. Moreno, I.**[1], Moreno, A.[1], Donoso, M.[1], Moraga, C.[1], Nebel, J.[2], Orellana, A.[1]. FONDAP Centro de Regulación del Genoma, Núcleo Milenio en Biotecnología Celular Vegetal,Centro de Biotecnología Vegetal, Facultad de Ciencias Biológicas, Universidad Andrés Bello[1], Faculty of Computing, Information Systems & Mathematics, Kingston University, London[2].

13 **Kinetic folding simulations of the knotted ribbon-helix-helix (RHH) protein VirC2 using a simplified structured-based model. Ramírez, C.**[1], Noel, J.K.[2], Baez, M.[1]. Department of Biology, Faculty of Sciences, University of Chile, Santiago, Chile[1], Center for Theoretical Biological Physics, University of California, San Diego, La Jolla CA 92093-0374, USA[2].

14 **The vitamin D receptor binds to nuclear matrix proteins in the presence and absence of vitamin D. Ruiz-Tagle, C.**[1], Nilo, R.[1], Orellana, A.[1], Montecino, M.[1]. FONDAP Center for Genome Regulation, Faculty of Biological Sciences, Universidad Andrés Bello, Santiago, Chile[1].

15 **Protein secretion by *Penicillium purpurogenum* under catabolite repression.** Eyzaguirre, J.[1], **Pérez, N.**[1], Navarrete, M.[1], Callegari, E.[2]. Universidad Andrés Bello[1], Universidad de Dakota del Sur, U.S.A.[2].

16 **Heterologous expression of phenylpropanoid pathway enzyme in bacteria.** Guzmán , L.[1], **Robles, C.**[1], Quintanilla, I.[1], González , P.[1], Aguilar, F.[2]. Laboratorio de Química Biológica. Instituto de Química. Pontificia Universidad Católica de Valparaíso[1], Laboratorio de Fotoquímica. Instituto de Química. Pontificia Universidad Católica de Valparaíso[2].

17 **Multiplex real-time PCR for the specific detection of Piscirickettsia salmonis. Calquín, P.**[1], Álvarez, C.[1], Valenzuela, K.[1], Cárcamo, J.G.[1], Avendaño-Herrera, R.[2], Yáñez, A.[1]. Instituto de Bioquímica y Microbiología, Universidad Austral de Chile[1], Laboratorio de Patología de Organismos Acuáticos y Biotecnología Acuícola, Universidad Andrés Bello[2].

18 **Characterization of proteins present in Sauvignon blanc wines and their role in protein haze formation. Lobos, F.**[1], De Bruijn, J.[2], Martínez-Oyanedel, J.[1]. Laboratory of Molecular Biophysics, Department of Biochemistry and Molecular Biology, Faculty of Biological Sciences, Universidad de Concepción, Chile[1], Department of Agroindustry, Universidad de Concepción, Chillán, Chile[2].

19 **Encapsulation of proteins on alginate-chitosan mixtures. Silva, H.**[1], Valenzuela, K.[1], Álvarez, C.[1], Sáez, M.[1], Pontigo, J.[1], Oliver, C.[1], Amthauer, R.[1], Yánez, A.[1]. Unversidad Austral de Chile[1].

20 **Evaluation of the effect on innate immunity of a new vaccine against *P.Salmonis.* Sáez, M.A.**[1], Espinoza, C.[2,3], Pontigo, J.P.[1], Valenzuela, K.[1], Silva, H.[1], Troncoso, J.[3], Romero, A.[1], Yáñez, A.[1]. Universidad Austral de Chile[1], Universidad de Concepción[2], EWOS Innovation[3].

21 **The mitochondrial SncmtRNA and the modulation of the expression profile of cell cycle genes. Oliveira-Cruz, L.**[1], Lyons, J.[2], Arraya, M.[1], Vidaurre, S.[3], Burzio, V.[3], Burzio, L.[3]. Andes Biotechnologies, Fundación Ciencia para la Vida[1], Andes Biotechnologies, University of California-San Francisco[2], Andes Biotechnologies, Fundación Ciencia para la Vida, Universidad Andrés Bello[3].

22 **LKB1 kinase is required for RHEB GTPase to activate AMP-activated protein kinase. Armijo, M.**[1], Pincheira, R.[1], Castro, A.[1]. Laboratorio de Transducción de Señales y Cáncer. Departamento de Bioquímica y Biología Molecular. Facultad de Ciencias Biológicas. Universidad de Concepción[1].

23 **Silencing of tumor gene *WIF1* in hereditary triple negative breast cáncer. Garrido, G.**[1], Alvarez, C.[1], Tapia, T.[1], Cornejo, V.[2], Fernández, W.[2], Camus, M.[3], Alvarez, M.[4], Carvallo, P.[1]. Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile[1], Unidad de Anatomía Patológica, Hospital San Borja Arriarán, Santiago, Chile[2], Centro de Cáncer, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile[3], Clínica Las Condes, Santiago, Chile[4].

24 **Knockdown of non-coding mitochondrial RNAs with antisense oligonucleotides induces changes in cell cycle-related proteins in normal and tumor cells. Fitzpatrick, C.**[1,2,3], Vidaurre, S.[1,2,3], Oliveira-Cruz, L.[1,2,3], Briones, M.[1,2,3], Burzio, V.[1,2,3], Villegas, J.[1,2,3,4], Burzio, L.[1,2,3,4]. Fundación Ciencia para la Vida[1], Andes Biotechnologies SA[2], Universidad Andrés Bello[3], GrupoBios SA[4].

25 **Genomic aberrations and BRCA1 silencing in hereditary triple negative breast tumors in Chilean women. Tapia, T.**[1], Sanchez, A.[1], Alvarez, C.[1], Cornejo, V.[2], Fernández, W.[2], Cruz, A.[3], Segovia, L.[3], Alvarez, M.[4], Camus, M.[5], Carvallo, P.[1]. Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile[1], Hospital San Borja Arriarán, Santiago, Chile[2], Hospital Barros Luco, Santiago, Chile[3], Clínica Las Condes, Santiago, Chile[4], Centro del Cáncer, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile[5].

26 **Effect of Temodal on the expression level of Major Vault Protein (MVP) in a Glioblastoma Multiforme cell line. Calderón, F.**[1], Barrientos, C.[1], Manriquez, R.[1], Quezada, C.[1], Yáñez, A.[1], Cárcamo, J.[1]. Universidad Austral de Chile[1].

27 **Detection of genomic rearrangements in the MLH1 and MSH2 genes in families with Hereditary Non Poliposis Colorrectal Cancer. Orellana, P.**[1,2], Alvarez, K.[1,2], Hurtado, C.[1], Church, J.[3], Lopez-Kostner, F.[1], Carvallo, P.[2]. Laboratorio Oncología y Genética Molecular, Unidad de Coloproctología, Clínica las Condes[1], Departamento Biología Celular y Molecular, Facultad Ciencias Biológicas, Pontificia Universidad Católica de Chile[2], Cleveland Clinic Foundation, USA[3].

28 **P53 tumor suppressor regulates the expression of the transcription factor SALL2 in response to genotoxic agents. Escobar, D.**[1], Castro, A.[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].

29 **The SALL2 transcription factor plays a role in cell proliferation. Sánchez, M.F.**[1], Catro, A.[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].

30 **M-RAS induces gen expression through a non canonic pathway in MCF-7 breast cancer cell. Campos, T.**[1], Armijo, M.[1], Rivera, A.[1], Pincheira, R.[1], Castro, A.[1]. Laboratorio de Transducción de Señales y Cáncer. Departamento de Bioquímica y Biología Molecular. Facultad de Ciencias Biológicas. Universidad de Concepción[1].

31 **Cathepsin L effect on cell cycle in Caco-2 cells. Pérez, V.**[1], Flaig, D.[1], Hermosilla, V.[1], Arrey, V.[1], Iribarren, C.[1], Leonardi, M.[1], Puchi, M.[1], Morin, V.[1] Department of Biochemistry and Molecular Biology, Faculty of Biological Sciences, Universidad de Concepción. Chile[1].

32 **Computer assisted design and chemical synthesis of apamin mimetic peptides to study their interaction with the human protein BIRC-5 expressed in *E. coli*. Carrasco, V.**[1], Guzmán, L.[1], Muñoz, E.[2], Guzmán, F.[3], Aguilar, L.[1]. Instituto de Química. Pontificia Universidad Católica de Valparaíso. Avenida Universidad #330. Campus Curauma, Valparaíso[1], Instituto de Física. Pontificia Universidad Católica de Valparaíso. Avenida Universidad #330. Campus Curauma, Valparaíso[2], Núcleo Biotecnología Curauma. Pontificia Universidad Católica de Valparaíso. Avenida Universidad #330. Campus Curauma, Valparaíso[3].

33 **CXCR3 and their spliced variants as tumour marker for Papillary Thyroid Cancer? Véliz, L.**[1,2], Vargas, S.[1], Bohmwal, K.[3], Catalán , T.[2], Kalergis, A.[2], Riedel, C.[3], González, H.[1]. Departamento de Cirugía Oncológica, Pontificia Universidad Católica de Chile, Santiago, Chile[1], Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile[2], Facultad de Ciencias Biológicas, Universidad Andrés Bello, Santiago, Chile[3].

34 **Effect of phorbol esters over nuclear cathepsin L in colon cancer cells. Hermosilla, V.**[1], Pérez, V.[1], Flaig, D.[1], Iribarren, C.[1], Leonardi, M.[1], Puchi, M.[1], Morin, V.[1] Department of Biochemistry and Molecular Biology, Faculty of Biological Sciences, Universidad de Concepción[1].

35 **Docking Studies of Coumarins Derivatives as lipoxygenase Inhibitors. Muñoz, A.**[1], García-Beltrán, O.[2], Mascayano, C.[1], Nuñez, M.T.[3,4], Cassels, B.K.[2,4], Fierro, A.[1,5]. 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 Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile[3], Instituto Milenio de Dinámica Celular y Biotecnología, Santiago, Chile[4], Instituto Milenio de Dinámica Celular y Biotecnología, Santiago, Chile[5]. Sponsored by F. Gonzalez Nilo.

36 **B-Metilated phenethylamines: the main interactions with monoamine oxidases and monoamine transporters. Zamora, R.**[1], Rebolledo, M.[2], Fierro, A.[1,3]. Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile[1], Facultad de Ciencias, Universidad de Chile, Santiago, Chile[2], Instituto Milenio de Dinámica Celular y Biotecnología, Santiago, Chile[3]. Sponsored by M. Imarai.

37 **Chalcones as monoamine oxidase inhibitors. Morales, N.**[1], Moya-Alvarado, G.[1], Dahech-Levenberg, P.[1], Pérez, E.[2], Caroli Rezende, M.[3], Fierro, A.[3]. Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile[1], Facultad de Química, Pontificia Universidad Católica de Chile, Santiago, Chile[2], Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile[3]. Sponsored by G. Zuñiga.

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CONFERENCES

Grabbing the Cat by the Tail: Discrete Steps and Inter-Subunit Coordination by a DNA Packaging Ring-ATPase. Bustamante, C.[1]. University of California, Berkeley[1].
As part of their infection cycle, many viruses must package their newly replicated genomes inside a protein capsid to insure its proper transport and delivery to other host cells. Bacteriophage phi29 packages its 6.6 mm long double-stranded DNA into a 42 nm dia. x 54 nm high capsid using a multimeric ring motor that belongs to the ASCE (Additional Strand, Conserved E) superfamily of ATPases. A number of fundamental questions remain as to the coordination of the various subunits in these multimeric rings. The portal motor in phi29 is ideal to investigate these questions. Using optical tweezers, we find that this motor can work against loads of up to ~57 picoNewtons on average, making it one of the strongest molecular motors ever reported. Interestingly, the packaging rate decreases as the prohead is filled, indicating that an internal pressure builds up due to DNA compression. The capsid pressure at the end of the packaging is ~6 MegaPascals, corresponding to an internal force of ~52 pN acting on the motor. We have identified where in the mechanochemical cycle the chemical energy of ATP is converted into mechanical work. Using ultra-high resolution optical tweezers, we have performed the first direct measurement of the step size of a translocating ring ATPase. What emerges is a surprising mechanism that involves a step size with a non-integer number of base pairs and that reveals an unexpected degree of coordination among the individual subunits that has not been proposed previously for a ring ATPase. A model of the motor's mechanochemical cycle is proposed based on these studies.

Light signalling networks in the control of plant development. Casal, J.J.[1,2]. Fundación Instituto Leloir[1], Universidad de Buenos Aires, Argentina[2].
Plants are exposed to a light environment that changes with the time of the day, the time of the year, the degree of cloudiness, the presence of neighbours and the position of plant organs above or below the soil surface. These changes represent both a challenge and an opportunity for plants; a challenge, because the aforementioned variations can generate physiological stress; an opportunity, because the anticipated perception of changes in the light environment provides information that helps plants to adjust to the incoming stressful conditions. Plants bear several photoreceptor types: the red and far-red light receptors phytochromes, the blue-light receptors cryptochromes, phototropins and members of the zeitlupe family, and the UV-B receptor UVR8. Downstream signalling involves a complex network where the photoreceptors repress negative regulators of photomorphogenesis. In darkness, the E3-ligase COP1 labels for destruction several transcription factors required for photomorphogenesis. In the light, the photoreceptors inactivate COP1 by direct interaction. Another mechanism of photoreceptor action involves their direct interaction with transcription factors. For instance, PIF bHLH transcription factors promote skotomorphogenesis in darkness. In the light, phytochromes migrate from the cytoplasm to the nucleus, where they bind PIF proteins and cause their phosphorylation and subsequent destruction. These pathways are connected to the hormone signalling pathways, creating complex networks that link light signals to physiological responses. Our current interest is to understand the mechanisms by which plants can attenuate the impact of non-informative fluctuations of the light environment and integrate different environmental signals controlling the shape and function of its body.

BioHydrogen production by fermentation in an artificial bacterial ecosystem. Interbacterial species communication by physical contact? Benomar, S.[1], Leroy, G.[1], Trably, E.[2], Giudici-Orticoni, M.[1], **Cárdenas, M.**[1]. Laboratoire de Bioénergétique et Ingénierie des Protéines, CNRS, 31 chemin Joseph Aiguier 13402 Marseille cedex 20 - France[1], Laboratoire de Biotechnologie de l'Environnement, INRA, Avenue des Etangs 11100 Narbonne, France [2].
In nature, fermentation of biomass is achieved by the action of consortia that involve different types of bacteria. Numerous microorganisms can produce hydrogen by dark fermentation", which opens the way for its industrial production. However, several scientific constraints remain to be addressed, and it is now necessary to acquire a better understanding of the metabolic interaction networks existing between producing and consuming microorganisms. Metabolic control analysis studies have shown that most biochemical pathways are controlled by demand, and that evolution appears to have favoured control mechanisms that decrease the concentrations of intermediates as much as possible. Thus, any genetic engineering that would disturb the regulation in order to optimize the production induces instability. Therefore, we have developed a multidisciplinary approach, consisting of the construction and study of a synthetic consortium of two bacteria that coexist in the wild: Clostridium acetobutylicum (cab), which produces H2 by fermentation of sugars, and Desulfovibrio vulgaris Hildenborough (DvH), which produces or consumes H2 according to its environment. We have investigated the effect on each other of the two bacteria, within the framework of the production of metabolites. Using different approaches, we show (i) the coexistence and growth of the two bacteria in the consortium, in spite the fact that DvH by itself is not able to grow on glucose; (ii) a higher H2 production by the consortium compared to isolated bacteria; (iii) changes in gene expression of cab. The type of interbacterial communication in the consortium will be discussed.

A new function for CPEB: nuclear processing of pre-mRNAs. Alessio, F.[1], **Méndez, R.**[1]. ICREA and Institute for Research in Biomedicine (IRB). Barcelona, Spain[1].
CPEB (for Cytoplasmic Polyadenilation Element Binding protein) was identified sixteen years ago as an RNA-binding protein that recognizes maternal mRNAs in the cytoplasm of Xenopus Laevis oocytes and directs their poly(A) tail elongation and translational activation during meiotic progression. Since then, CPEB has been shown to regulate the translation of hundreds of mRNAs in both somatic and germ cells and to drive events as diverse as learning and memory, cell cycle progression and tumor development. Now, we have found that the cytoplasm and the translational regulation is only part of the life of a protein that moonlights as a nuclear factor responsible for the pre-mRNA processing of the same mRNAs that, later, is going to regulate at the translational level. Thus, CPEB is a nucleocytoplasmic shuttling protein that recognizes the same cis-acting element in the cytoplasmic mature mRNA as in the nuclear pre-mRNA, recruiting the cleavage and polyadenylation machinery that mediates both the cytoplasmic polyadenylation and the nuclear pre-mRNA cleavage and polyadenylation at specific polyadenylation sites. In turn, at least in some cases, this affects alternative splicing of the CPEB regulated transcripts. This is a new function for CPEB, where hundreds of mRNAs are regulated by alternative processing in the nucleus in a coordinated manner and associated with cell cycle and tumor development. A global model for the regulation of gene expression by the CPEB family of proteins in cell cycle and cell differentiation will be presented.

SYMPOSIA

Development of bioinformatics tools to assist the inference of sequence-structure-function relationships in small RNAs. Norambuena, T.[1,2], **Melo, F.**[1,2]. Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Alameda 340, Santiago, Chile [1], Molecular Bioinformatics Laboratory, Millennium Institute on Immunology and Immunotherapy, Santiago, Chile[2].
Over the recent years, the vision that RNA simply serves as information transfer molecules has dramatically changed. The study of the sequence/structure/function relationships in RNA is becoming more important. As a direct consequence, the total number of experimentally solved RNA structures has dramatically increased and new computer tools for predicting 3D RNA structure from sequence are rapidly emerging. Therefore, new methods for assessing the accuracy of RNA structure models are clearly needed. Here we describe an all-atom knowledge-based potential for the assessment of RNA 3D structures. We have benchmarked our new potential, called RASP, with two different decoy datasets composed of near-native RNA structures. In one of the benchmark sets, RASP was able to rank the closest model to the X-ray structure as the best and within the top 10 models for ~93% and ~95% of decoys, respectively. Based on a recently released benchmark dataset that contains hundreds of 3D models for 32 RNA motifs with non-canonical base pairs, RASP scoring function compared favorably to ROSETTA FARFAR force field in the selection of accurate models. Finally, using the self-splicing group I intron and the stem-loop IIIc from hepatitis C virus internal ribosome entry site as test cases, we show that RASP is able to discriminate between known structure-destabilizing mutations and compensatory mutations. RASP can be readily applied to assess all-atom or coarse-grained RNA structures and thus should be of interest to both developers and end-users of RNA structure prediction methods.

In vitro studies of the molecular mechanisms involved in miRNA repression. Ricci, E.P.[1,2,3], Limousin, T.[1,2,3], Soto Rifo, R.[1,2,3], Decimo, D.[1,2,3], **Ohlmann, T.**[1,2,3]. Ecole Normale Supérieure de Lyon, Unité de Virologie Humaine, IFR 128, Lyon, F-69364 Franch[1], Inserm, U758, Lyon, F-69364 France[2], Université de Lyon[3].
microRNAs (miRNAs) are small non coding RNAs that regulate gene expression by mediating translational repression, deadenylation and decay of targeted messenger RNAs (mRNAs). Although recent papers have identified how miRNA regulate deadenylation and decay, their impact on translation remains controversial. Here we have first designed an in vitro system based on the rabbit reticulocyte lysate to study miRNA repression. This systems enables to focus on the effects of miRNAs on translation with no impact on mRNA stability. Thus, by using this novel sytem, we have studied the effect of endogenous miR-451, the most abundant miRNA in the reticulocyte lysate, on translation of various constructs. By using various constucts bearing natural 5' untranslated regions (5'UTR), we first showed that the magnitude of miRNA repression was independent from the translational efficiency of the mRNA. Such a result allowed us to monitor and compare miRNA activity through the screening of specific inhibitors of various stages of translation. This showed that miR-451 repression exclusively takes place at the level of translation initiation with no effect on ribosomal subunit joining, elongation or termination. Further investigation into the molecular mechanism showed that miR-451 interferes specifically with 43 S ribosomal scanning and requires the Poly(A) binding protein independently from its association with eIF4G. This work was supported by ANRS, FRM and INSERM.

Loading the ribosome on the mRNA, the intriguing case of HIV. Chamond, N.[1]. Deforges, J.[1]. Brossard, A.[1], Ulryck, N.[1], **Sargueil, B.**[1]. UMR 8015, Laboratoire de cristallographie et RMN Biologique, Université Paris Descartes, 4 avenue de l'Observatoire, 75270 Paris Cedex 06. Francia[1].
Viruses hijack their host machineries for all steps of their cycle, notably for replication, transcription and translation. This is often driven by the ability of the viral encoded proteins to interact with cellular proteins. Viral RNA is also able to adopt three dimensional structures that can divert cellular function. The genomic RNA of the Human Immunodeficiency Virus provides very good examples of this. It comprises a long 5'untranslated region (5'UTR) followed by the gag/pol open reading frame. The 5'UTR of this RNA folds in structures required for different steps of the virus cycle. Among its diverse functions it recruits internally the translation initiation complex, and this promotes Gag translation in G2/M phase of the cell cycle. We then explore the potential for the gag open reading frame to be endowed with other functions than specifying the amino-acid sequence. We found that gag coding sequence was also able to recruit the translation initiation complex. Characterizing the molecular mechanism involved in this phenomenon we found that the coding region folds in a structure that tightly binds the small subunit of the ribosome and a protein complex essential for translation, eiF3. We have reconstituted in vitro the sequence of events that leads from this initial ternary complex to the active initiation complex. Those results will be discussed in regards to the physiology of the virus, and the strategy used by other viruses to hijack the translation machinery.

The regulatory mechanism of phosphofructokinase-2 from *E. coli* by ATP: allosteric transitions characterized by X-ray crystallographic, biochemical and bioinformatics methods. **Baez, M.**[1]. Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago[1].
Allosteric inhibition by MgATP is a regulatory feature of the phosphofructokinase isoenzymes from *E. coli* (Pfk-1 and Pfk-2). Under gluconeogenic conditions, the loss of this regulation causes substrate cycling of fructose-6-P and futile consumption of ATP delaying growth. Pfk-2 is a dimer containing a single allosteric site for MgATP and active site per subunit, but differently from classical allosteric effectors, typically localized away from the active site, the allosteric ATP makes contacts with the phosphates from the catalytic ATP. This suggests a direct modification of the active site environment upon allosteric binding into each monomer. However, long range communication between active and allosteric sites localized in different subunits are also plausible, since some side chains coming from the partner subunit participate in both sites. In order to understand the kinetic features of the MgATP induced inhibition, the conformational changes associated with the allosteric transitions induced by the nucleotide will be presented through the X-ray structures of Pfk-2 obtained in different states of ligation, with fructose-6-P and MgATP. Moreover, kinetics analyses performed with several hybrid dimers, containing either single intact allosteric or single intact fructose-6-P binding sites, will be shown. These results, together with an analysis of correlated side chain movements between and within each subunit, give a global view of the allosteric mechanism of Pfk-2. Fondecyt 1090336.

Communication in phycobilisomes from *Gracilaria chilensis*. **Bunster, M.**[1]. Depto. Bioquimica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción[1].
Protein surfaces are the key element for protein protein recognition, nevertheless protein communication must initiate response such as electron transfer, tissue formation, inhibition of enzymes or energy transfer among others. In the phycobilisome, whose function is to harvest light and transfer energy towards the photosynthetic reaction centers, the correct packing of the components of this gigantic protein complex, allows the correct position of their chromophores allowing the most efficient energy transfer system known in nature.Phycobilisomes are formed by phycobiliproteins(PBP) and linkers(L). The basic unit of a phycobiliprotein is the heterodimer $\alpha\beta$, which oligomerizes to form ring shaped $(\alpha\beta)_6$. Each PBP differ in sequence and in phycobilin composition, property that gives each one, their spectroscopic characteristics. As nature works with logic, the architecture of the complex favors its function. A core of Allophycocyanin and rods of Phycocyanin and Phycoerythrin that radiate from the core. Each phycobiliprotein unit transfers energy to the next with a rate transfer that depends on the length of the conjugate system of the phycobilins, their geometry, the distance between them, the magnitude of the overlapping absorption and emission spectra, the angle between the transition dipole moments and on the protein environment. DIUCUdeC 211.037.012-1.0, FONDECYT N 108.0267.

Functional consequences of intrinsic disorder in the NFκB-IκB interaction. **Komives, E.**[1]. Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla CA 92093-0359, USA[1].
The NFκB transcription factor family controls a myriad of cellular functions including growth and differentiation and stress responses. Our work focuses on the NFκB(RelA/p50) heterodimer, which is more abundant in most cell types. In resting cells, NFκB is sequestered in the cytoplasm because its nuclear localization signal (NLS) is masked by binding to an inhibitor protein, IκBα. Crystal structures of NFκB bound to DNA and to IκBα are available. Truncation of both proteins and measurement of binding affinities in vitro showed that the binding affinity of NFκB to IκBα is extremely tight (K_D 40 pM), and the binding energy is concentrated at the ends of the elongated protein-protein interface. IκBα binds to NFκB via its ankyrin repeat (AR) domain and biophysical characterization of this domain showed that AR1-AR4 are well-folded while AR5-AR6 are intrinsically disordered, but fold on binding to NFκB. The half-life of the free IκBα is less than 10 minutes in cells, and it is degraded by the proteasome in an ubiquitin-independent manner. This rapid degradation depends on the disordered AR5-AR6, but not on the overall stability of the domain. Pre-folding AR6 significantly lengthens the intracellular half-life, and the half-life of NFκB-bound IκBα is many hours. The very tight binding affinity of NFκB results from coupled folding and binding events at either end of the interface. The disorder in AR5-AR6 is also involved in facilitating dissociation of NFκB from the DNA transcription sites. Funding: NIH P01 GM071862.

B cells in Trypanosoma cruzi infection: role as antibody-secreting cells and as cytokine producers. Gruppi, A.[1]. Universidad Nacional de Córdoba, Argentina[1].

Acute infection with Trypanosoma cruzi, the etiologic agent of Chagas disease, results in parasitemia, polyclonal B cell activation (associated with hypergammaglobulinemia), and massive germinal center (GC) and extrafollicular (EF) B cell response at the peak of infection. EF foci are evident since day 3 post-infection (p.i.) and they mainly provide IgM. GCs are remarkable since day 8 p.i. At the peak of parasitemia, CD138+ B220+ plasma cells in EF foci, in red pulp and in T zone expressed IgM and all the IgG isotypes. Instead of the substantial B cell response, parasite-specific IgG isotypes could be detected in sera only after 18 days p.i.; therefore most of the Abs produced by splenic cells were non-parasite specific. Bone marrow of infected mice presents a strong reduction in CD138+B220+ cells in comparison to normal mice, and then the spleen appears as the most important lymphoid organ that lodges plasma cells. B and plasma cells from T. cruzi infected mice are a significant source of IL-10, IL-6, TNF-alpha and other cytokines that condition T cell response. Absence of mature B cells (uMT mice) affects cytokine production and different T cell population in T cruzi infected mice. Results indicate that in T.cruzi infection B and plasma cells not only provide Abs but they also secrete cytokines and regulate cellular response.

Immunity to flagellated bacteria. McSorley, S.[1]. University of California Davis, Center for Comparative Medicine[1].

Pathogens can substantially alter gene expression within an infected host depending on metabolic or virulence requirements in different tissues, however, the effect of these alterations on host immunity are unclear. We have visualized multiple CD4 T cell responses to temporally expressed proteins in Salmonella-infected mice in order to examine how temporal expression affects adaptive immunity. Flagellin-specific CD4 T cells expanded and contracted early, differentiated into Th1 and Th17 lineages, and were enriched in mucosal tissues after oral infection. In contrast, CD4 T cells responding to Salmonella Type-III Secretion System (TTSS) effectors steadily accumulated until bacterial clearance was achieved, primarily differentiated into Th1 cells, and were predominantly detected in systemic tissues. Thus, pathogen regulation of antigen expression plays a major role in orchestrating the expansion, differentiation, and location of antigen-specific CD4 T cells in vivo.

Impact of virulence genes acquired by lateral gene transfer in the immune response against Salmonella. Bueno, S.[1,2]. Millennium Institute on Immunology and Immunotherapy[1], Facultad de Ciencias Biológicas. P. Universidad Católica de Chile.[2].

Salmonella are Gram-negative bacteria and the etiological agent of several diseases in humans. The ability of Salmonella to cause a systemic disease in the host is due to its capacity to survive and replicate inside eukaryotic cells, especially within epithelial and phagocytic cells. Moreover, these bacteria prevent the activation of the adaptive immune response by its capacity to interfere with dendritic cell (DCs) function. Virulence factors that allow Salmonella to cause a systemic disease are located in pathogenicity islands, which are DNA fragments acquired by lateral gene transfer. In Salmonella there are two kinds of pathogenicity islands: stable and unstable. We have recently shown that genes encoded in both kinds of pathogenicity islands are required by these bacteria to interfere with DC function. On the one hand, we have shown that genes found in a stable pathogenicity island of Salmonella are required for the bacteria to prevent the capture by DCs. On the other hand, genes found in unstable pathogenicity island are required by Salmonella to survive inside these cells. Interestingly, we have observed that the capacity of unstable pathogenicity islands to excise from the bacterial chromosome and be kept as an extrachromosomal element is increased when Salmonella reside inside DCs. Our results suggest that: 1. Stable and unstable pathogenicity islands of Salmonella are required by these bacteria to interfere with the function of dendritic cells and 2. Unstable pathogenicity islands are able to sense specific conditions in DCs that promote their own excision.

Coupling transcription regulation with DNA replication by the DnaA protein. Sclavi, B.[1]. CNRS, ENS Cachan, France[1].

At growth rates faster than 60 minutes the rate of DNA replication in Escherichia coli is increased mainly by an increase in the number of replication forks resulting from overlapping rounds of DNA replication. The DnaA protein plays a key role in the regulation of genome stability by directly binding and activating the origin of replication and by its role as a transcription factor. DnaA expression is known to be growth rate dependent and subject to negative auto-regulation. We have carried out a quantitative characterization of transcription regulation of the dnaA promoter region as a function of growth rate and temperature in order to obtain an accurate description of one of the main regulatory networks required for genome stability in bacteria. We provide direct evidence that in addition to negative auto-regulation, the dnaA promoter is also positively auto-regulated and that the effect of auto-regulation is independent of both growth rate and temperature. The temperature dependence of DnaA-ATP activity however suggests that increased titration to its sites on the genome can compensate for the induction of its expression required for origin activation at low temperatures. Finally we have shown that despite its similarities to stable RNA promoters, expression fromdnaAP2 begins to increase mainly at growth rates greater than 1 doubling per hour, proportionally to the increase in the rate of DNA replication and independently of the presence of positive and negative auto-regulation.

Innovative Strategies For Teaching Biochemistry Herrera, R. Moya-Leon, M.A.[1]. Instituto Biologia Vegetal y Biotecnologia, Universidad de Talca[1].
Teaching biochemistry in the current state of science and society requires a special motivation for learning, especially for students of degrees other than Biochemistry. The traditional way of teaching, based on the teacher-student relationship, mostly unidirectional, does not fulfil the needs imposed in this era. Considering the current situation, University students require new abilities in their training and the use of computers can constitute a place for discovery and research, enabling the experience of new and diverse situations, under rules and characteristics outlined according to the objectives aimed by the teacher. The design of teaching material for undergraduate students who take biochemistry courses as complementary subject on their careers should be seen as opportunity to complement theoretical aspect on the current courses. Three different approaches could be used: (I) a description of the basic concepts, like in a book but using dynamics figures. (II) Modelling proteins indicating key motifs at the three-dimensional structure and residues where inhibitors can be attach. And (III) elaborating active quizzes where students can be drive on their learning. Building knowledge based on practical experience can improve student?s competence on basic science and the learning process can be complemented in the use of dynamics models.

Experimental Biochemistry Simulation. Claude, A.[1], von Chrismar Parejo, A.M.[1]. Instituto de Bioquímica y Microbiología, Universidad Austral de Chile[1].
We have endeavored to recover a one-on-one educational model through the utilization of simulated biochemistry experiments in massive courses. This approach, using information and communication technologies aims to improve the learning and comprehension of complex, difficult to visualize, biological concepts. Under this paradigm, students can learn at their own pace and with all the reiterations required, with an approach that avoids flooding them with new concepts under pressure and limited time, but rather reinforces the creation of logical processes to resolve problems. Our virtual experimental biochemistry modules simulate standard biochemical laboratory teaching units as an individual experience, maintaining a traditional organizational structure. Unlike regular laboratory experimental units with a large numbers of students and limited hands-on opportunities, users recover an individual experience in simulated experiments, while the instructors recover a large measure of control over the teaching process. Our system carefully monitors the student's activities to avoid cheating, identity theft and copying of results. Our successful experience using this approach has led to the development of an open, community-driven support platform and specialized software that allows teachers without previous experience and no programming knowledge to develop their own virtual laboratory modules. Currently we aim to expand usage of this system through the creation of an international network of users that share their modules and usage experience.

New strategies in the teaching of biochemistry at the University of Concepción. Martínez Oyanedel, J.[1], Bruna, C.[1], Bunster, M.[1]. Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción[1].
The new paradigms of higher education require new teaching strategies to meet the learning objectives of Biochemistry courses. In our department there have been some early educational innovations through the incorporation of topics of protein structure in web page, which then evolved into interactive java applications based on the use of CHIME, and is currently being adapted to Jmol. Another tool used is the PROTLAB software, which is a simulator of protein purification. Recently have added new activities to motivate the study of Biochemistry, especially first years student: Art and Biochemistry where the students express some of the biochemistry concepts through artistic expression. For senior student, have been designed activities to apply the basic knowledge of biochemistry to the explanation of everyday events for the general public, through the generation of a Wiki in a simple language. Debates activities have also been established for discussion of topics related to biochemistry. All activities have a greater acceptance of students, although the effect on learning outcomes is ambiguous.

The Information Technologies and the Teaching of Biochemistry. Galembeck, E.[1]. Depto. de Bioquímica - IB - UNICAMP, Campinas - SP - Brasil[1].
The new information and communication technologies (NICTs) have changed not only in the way that the information is delivered, but also the way that the knowledge is built. These technologies have been used widely from the most traditional to the most creative ways both in research and education. Some of the new technologies uses can be an alternative to the not so new ones such as using Internet to deliver texts, audios and videos, but they can also allow new applications that were not possible a few years ago. In this presentation four relevant aspects related to the uses of the new information and communication technologies in Biochemistry Education will be discussed: 1) NICTs promote new ways of learning, but require new things to be learned; 2) Which biochemistry contents and skills can be learned using NTICs and which ones cannot; 3) How can we take advantage of the information stored in online databases; 4) What are the new assessment approaches that the new technologies allow. The discussion concerning the four topics presented above will be illustrated with examples of the systems that are already implemented, and with the possibilities where the current technologies may be used.

NEW MEMBERS SESSIONS

Sorting of the Alzheimer's disease amyloid precursor protein mediated by the AP-4 complex. Mardones, G.[1,2], Burgos, P.[1,2], Rojas, A.[3], da Silva, L.[2], Prabhu, Y.[2], Hurley, J.[3], Bonifacino, J.[2]. Instituto de Fisiología, Facultad de Medicina, Universidad Austral de Chile, Valdivia[1], Cell Biology and Metabolism Program, NICHD[2], Laboratory of Molecular Biology, NIDDK, NIH, Bethesda, MD, USA[3].
Adaptor protein 4 (AP-4) is the most recently discovered and least well-characterized member of the family of heterotetrameric adaptor protein (AP) complexes that mediate sorting of transmembrane cargo in post-Golgi compartments. Herein, we report the interaction of an YKFFE sequence from the cytosolic tail of the Alzheimer's disease amyloid precursor protein (APP) with the mu4 subunit of AP-4. Biochemical and X-ray crystallographic analyses reveal that the properties of the APP sequence and the location of the binding site on mu4 are distinct from those of other signal-adaptor interactions. Disruption of the APP-AP-4 interaction decreases localization of APP to endosomes and enhances gamma-secretase-catalyzed cleavage of APP to the pathogenic amyloid-beta peptide. These findings demonstrate that APP and AP-4 engage in a distinct type of signal-adaptor interaction that mediates transport of APP from the trans-Golgi network (TGN) to endosomes, thereby reducing amyloidogenic processing of the protein.

Characterization of the binary complex [SdiA/ acyl-HSL] in the transcription of the *ftsQAZ* operon. There is a correlation between quorum sensing and cell division? Gallardo, M.J.[1,2], Guiliani, N.[1], Monasterio, O.[1], Prevelige, P.[3], Bustamante, C.[4]. Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile[1], CEFOP, Facultad de Física y Matemáticas, Universidad de Concepción, Concepción, Chile[2], Department of Microbiology, University of Alabama at Birmingham, Birmingham, USA[3], University of California, Berkeley, California, USA., Howard Hughes Medical Institute, Berkeley, California, USA[4].
Cell communication mechanism in bacteria is known as *Quorum Sensing*. In the bacterium *Escherichia coli* a protein homologous to type R transcriptional regulators was identified. This transcriptional regulator, SdiA, was associated to the transcription of *ftsQAZ* operon. An increment of the solubility was observed in the soluble fraction with AHL. In order to explain this effect and how AHL influences the binding of SdiA to DNA the effect of AHL on protein dimerization was characterized. The induction of a conformational change associated to the binding of the ligand and its dimerization was determined measuring AHL binding to SdiA. The affinity was in the nM range, indicating that quenching is due to a change led by AHL binding that it is associated to dimerization. For this reason the effect of AHL on the quaternary structure of the protein was evaluated. The results showed that the protein in absence of AHL is mainly a monomer and in the presence of AHL the dimer was induced. To identify the protein regions involved in the conformational changes isotopic exchange was used. It was observed a significant protection to exchange in the N-terminal domain and in the DNA binding domain. It was observed a high affinity of the protein to promoter sequence *ftsQP2*, results that were confirmed by visualization through AFM. There was no effect of the AHL on the binding of SdiA to DNA. FONDECYT 1095121.

Insulin signalling increases mitochondrial metabolism by promoting mitochondrial fusion. Parra, V.[1], Verdejo, H.[1,2], Troncoso, R.[1], Kuzmicic, J.[1], del Campo, A.[1], Pennanen, C.[1], Lopez-Crisosto, C.[1], Chiong, M.[1], Zorzano, A.[3], Lavandero, S.[1]. FONDAP Center for Molecular Studies of the Cell, Facultad de Ciencias Químicas y Farmacéuticas / Facultad de Medicina, Universidad de Chile, Santiago 8380492, Chile.[1], División de Enfermedades Cardiovasculares, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile.[2], Institute for Research in Biomedicine (IRB Barcelona), Baldiri i Reixac, 10, 08028 Barcelona, Spain.[3].
Mitochondria form a dynamic network that undergoes fission and fusion events, the latter controlled by the proteins mitofusin (Mfn1/2) and Opa-1. Although the disruption in their expression impairs mitochondrial metabolism, it is not clear if the physiological regulation of mitochondrial function directly involves changes in mitochondrial dynamics. Insulin is a key regulator of mitochondrial metabolism in cardiomyocytes and the goal of this study was to determine if insulin regulates mitochondrial metabolism affecting mitochondrial morphology. Cultured cardiomyocytes were treated with insulin for 0 - 24 h and mitochondrial three-dimensional images were obtained by confocal microscopy. 3 h of insulin promoted mitochondrial fusion as evidenced by an increase in mitochondrial volume (+156%) and a reduction in the mitochondrial number (-60%). These changes were associated with an increase in Opa-1 protein (+3.7 folds), mitochondrial membrane potential (+21%), intracellular levels of ATP (+28%) and oxygen consumption rate (+19%) in *in vitro* and *in vivo* models. Opa-1 or Mfn2 adenoviral knock-down of cardiomyocytes prevented all the insulin-induced changes in mitochondrial morphology and function. Finally, the inhibiton of Akt and mTORc1 prevented the mitochondrial fusion, metabolic boost and the Opa-1 increase observed after insulin treatment, suggesting an active role of these proteins controlling mitochondrial dynamics. All these data indicate for first time that insulin acutely regulates mitochondrial metabolism through a mechanism that is dependent upon increased mitochondrial fusion. VP, HV, RT, JK, AdC, CP, CL, are funded by CONICYT. FONDAP 1501006, FONDECYT 1080436, MECESUP UCH0802.

Activity of the human immunodeficiency virus type 1 cell cycle-dependent internal ribosomal entry site is modulated by IRES trans-acting factors. Vallejos, M.[1], Deforges, J.[2], Plank, T.M.[3], Letelier, A.[1], Ramdohr, P.[1], Abraham, C.G.[4], Valiente-Echeverría, F.[1], Kieft, J.S.[5], Sargueil, B.[2], Lopez-Lastra, M.[1]. Laboratorio de Virología Molecular, Instituto Milenio de Inmunología e Inmunoterapia, Centro de Investigaciones Médicas, Facultad de Medicina, Pontificia Universidad Católica de Chile[1], CNRS UMR 8015, Laboratoire de cristallographie et RMN Biologique, Universite Paris Descartes, Paris Cedex 06, France[2], Department of Biochemistry and Molecular Genetics[3], Department of Microbiology[4], Department of Biochemistry and Molecular Genetics and Howard Hughes Medical Institute[5], University of Colorado Denver School of Medicine, CO, USA.
The full-length mRNA of the human immunodeficiency virus type 1 (HIV-1), member of *Retroviridae* family and AIDS etiological agent, harbors an internal ribosomal entry site (IRES) element within its 5' untranslated region (5'UTR) that allows the recruitment of the translational machinery. This IRES drives the synthesis of Gag structural protein and is active during the G2M cell cycle phase. The molecular mechanisms governing IRES function are not understood. Here we show that translation initiation mediated by the HIV-1 IRES requires the participation of trans-acting cellular factors other than the canonical translational machinery. To gain insight into structure-function relationship of the HIV-1 IRES, we modeled the RNA structure of the HIV-1 5' leader. We used standard' chemical and enzymatic probes and an "RNA SHAPE" analysis to model the structure of the HIV-1 5' leader and we show, by means of a footprinting assay, that G2M extracts provide protections to regions previously identified as crucial for HIV-1 IRES activity. The introduction of mutations designed to alter the structure of the HIV-1 5' leader did not significantly affect IRES activity suggesting that the structure-function relationship within the HIV-1 IRES is not as rigid as has been described for other viral IRESes. Finally, we used a proteomic approach to identify cellular proteins within the G2M extracts that interact with the HIV-1 5' leader. Together, data show that HIV-1 IRES-mediated translation initiation is modulated by cellular proteins. This work was supported by Fondecyt 1090318.

Array CGH genomic profile of hereditary breast cancer tumors. Identification of tumor suppressor genes in deleted regions, determination of promoter hypermethylation and their protein expression in tumor biopsies. **Álvarez, C.**[1], Tapia, T.[1], Cornejo, V.[2], Aravena, A.[3], Fernández, W.[2], Camus , M.[4], Alvarez, M.[5], Maass, A.[3], Carvallo, P.[1]. Depto de Biología Celular y Molecular, P. Universidad Católica de Chile, Santiago[1], Unidad de Anatomía Patológica, Hospital San Borja Arriarán, Santiago[2], Centro de Modelamiento Matemático, Universidad de Chile [3], Centro de Cáncer, Fac de Medicina, P. Universidad Católica de Chile, Santiago[4], Clínica Las Condes, Santiago, Chile[5].

Breast cancer, as other cancer types, is a genetic disease caused by sequential accumulation of mutations and genomic alterations involving tumor suppressor genes and oncogenes. We analyzed by array-CGH tumor DNA from 52 patients with hereditary breast cancer: 3 *BRCA1*, 4 *BRCA2* and 45 from patients with no identified mutations (BRCAX). Our analysis revealed that *BRCA1* mutated tumors have different genomic alterations compared to *BRCA2* tumors. BRCAX tumors showed frequent deletions at: 1q21.3 (20%), 1p31.1 and 9q33.1 (18%); and frequent amplifications at 1q23.1 (20%), followed by 1q21.1 and several gains in chromosome 19 (18%). All genes involved in the deleted or amplified regions were compared with gene expression data in the ONCOMINE database finding correspondence among genomic alterations and gene expression. Based on these results, we selected 3 tumor suppressor genes for promoter methylation and protein expression analyses: *RASSF1A, SLIT2* and *WIF1*. Methylation analysis through MS-PCR revealed that *RASSF1A* promoter was hypermethylated in 67% of hereditary tumors, and significantly associated to loss of expression (p=0.007, OR 9.6, CI 1.77-52.19). *WIF1* promoter was hypermethylated in 68% of tumors with a significant association to loss of expression (p=0.042, OR 6.4, CI 1.155-35.45). Finally, *SLIT2* was found as the most frequently hypermethylated promoter among tumors (80%) showing loss of expression in 90% of these tumors. Our results indicate that silencing of *RASSF1A, SLIT2* and *WIF1* through promoter hypermethylation may have an important role in hereditary breast tumor development. Fondecyt 1040779 y 1080595.

Inhibition of Cyclin-Dependent Kinase 5 but Not of Glycogen Synthase Kinase 3-b prevents Neurite Retraction and Tau Hyperphosphorylation Caused by Secretable Products of Human T-Celi Leukemia Virus Type I-Infected Lymphocytes. **Maldonado, H.**[1,2], **Ramirez, E.**[3], **Utreras, E.**[4], **Pando, M.**[1], **Kettlum, A.**[1], **Chiong, M.**[1], **Kulkarn, A.**[4], **Collados, L.**[1], **Puente, J.**[1], **Cartier, L.**[5], **Valenzuela, M.**[1] Departamento de Bioquímica y Biología Molecular, Universidad de Chile[1], Laboratorio de Comunciaciones Celulares, Universidad de Chile[2], Programa de Virología, Universidad de Chile[3], Functional Genomics Section, Laboratory of Cell and Developmental Biology, NIDCR, NIH[4], Departamento de Ciencias Neurológicas, Universidad de Chile[5].

Human T-cell leukemia virus type I (HTLV-I)-associated myelopathy/ tropical spastic paraparesis (HAM/TSP) is a neurodegenerative disease characterized by selective loss of axons and myelin in the corticospinal tracts. Previous work showed tau hyperphosphorylation at T181 in cerebrospinal fluid of HAM/TSP patients. Similar hyperphosphorylation occurs in SH-SY5Y cells incubated with supernatant from MT-2 cells (HTLV-I-infected lymphocytes secreting viral proteins, including Tax) that produce neurite shortening. Tau phosphorylation at T181 is attributable to glycogen synthase kinase 3-b (GSK3-b) and cyclin-dependent kinase 5 (CDK5) activation. We investigate the effect of secretable HTLV-I products, like Tax protein, on neurite retraction in the SH-SY5Y model associates with concurrent changes in other Tau hyperphosphorylable residues. Tax neutralization on MT-2 supernatat with anti-Tax antibody prevented the neurite shortening. Threonine 181 turned out to be the only tau hyperphosphorylated residue. We also evaluate the role of GSK3-b and CDK5 in this process by using specific kinase inhibitors (LiCl, TDZD-8, and roscovitine) or siRNA duplex. LiCl or TDZD-8(GSK3-b inhibitors) was unable to prevent MT-2 supernatant-mediated neurite retraction and also that neither Y216(activating) nor S9(inactivating) phosphorylations were changed in GSK3-b. On the other hand, the CDK5 involvement in tau phosphorylation was confirmed by both the increase in its enzymatic activity and the absence of MT-2 neurite retraction in the presence of roscovitine or CDK5 siRNA transfection. The results of this study lead us to conclude that CDK5 or Tax inhibitors may be therapeutically useful in HAM/TSP. Fondecyt 1080396.

Effects of Knock-Down of the Antisense Noncoding Mitochondrial RNAs on Cell Cycle and Viability of Normal and Tumor Cells. **Burzio, V.A.**[1,2,3], Vidaurre, S.[1,2,3], Fitzpatrick, C.[1,2,3], Oliveira-Cruz, L.[1,2,3], Echenique, J.[1,2], Briones, M.[1,2,3], Villegas, J.[1,2,3,4], Burzio, L.O.[1,2,3,4]. Fundacion Ciencia para la Vida[1], Andes Biotechnologies SA[2], Universidad Andrés Bello[3], GrupoBios SA[4].

The family of non-coding mitochondrial RNAs (ncmtRNAs) comprises sense (SncmtRNA) and antisense (ASncmtRNA) members which are differentially expressed according to proliferative status: normal proliferating cells proliferation express both transcripts, tumor cells down-regulate the ASncmtRNA and resting cells down-regulate both (Villegas et al, 2007; Burzio VA et al, 2009). Knock-down of the ASncmtRNA with oligonucleotides (ODNs) induces selective apoptotic death of tumor cells, leaving normal cells unaffected. Death is preceded by decrease in proliferatiion, reflected in diminished expression of cell cycle proteins cyclinD1, PCNA and E2F1. In normal cells, similar effects are observed with ODN against SncmtRNA, in a reversible manner. These results suggest a role for the ncmtRNAs in cell cycle progression. Using a murine melanoma model, we show that knock-down of ASncmtRNA not only induces death, but also abolishes tumorogenicity of residual live tumor cells, shown by their inability to form colonies in soft agar and tumors in mice. An in vivo therapy assay shows that tumors in mice can be treated with specific ODNs. These results suggest that this knock-down treatment targets the cancer stem cells as well as the general tumor cell population. Fondecyt-1110835, PFB-16, UNAB-DI-31-09/R.

Distributed Structures Underlie Gating Differences between the Kin Channel KAT1 and the Kout Channel SKOR. Riedelsberger, J.[1], Sharma, T.[1], **Gonzalez, W.**[2], Gajdanowicz, P.[1], Morales-Navarro, S.E.[2], Garcia-Mata, C.[3], Mueller-Roeber, B.[1], Gonzalez-Nilo, F.D.[2], Blatt, M.R.[3], Dreyer, I.[1]. Universitat Potsdam, Germany[1], Universidad de Talca, Chile[2], University of Glasgow, UK[3].

The family of voltage-gated (Shaker-like) potassium channels in plants includes both inward-rectifying (Kin) channels that allow plant cells to accumulate K+ and outward-rectifying (Kout) channels that mediate K+ efflux. Despite their close structural similarities, Kin and Kout channels differ in their gating sensitivity towards voltage and the extracellularK+ concentration. We have carried out a systematic program of domain swapping between the Kout channel SKOR and the Kin channel KAT1 to examine the impacts on gating of the pore regions, the S4, S5, and the S6 helices. We found that, in particular, the N-terminal part of the S5 played a critical role in KAT1 and SKOR gating. Our findings were supported by molecular dynamics of KAT1 and SKOR homology models. In silico analysis revealed that during channel opening and closing, displacement of certain residues, especially in the S5 and S6 segments, is more pronounced in KAT1 than in SKOR. From our analysis of the S4?S6 region, we conclude that gating (and K+-sensing in SKOR) depend on a number of structural elements that are dispersed over this 145-residue sequence and that these place additional constraints on configurational rearrangement of the channels during gating.

ORAL SESSIONS

STRUCTURE AND FUNCTION OF MACROMOLECULES

Standardized comparison of structural alignments of catalytic domains in DNA polymerases from different methods. Slater, A.W.[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, Alameda 340, Santiago, Chile.[2].
Structural comparison of functional protein domains is a powerful technique to generate accurate knowledge about sequence/ structure/function relationships in protein families that contain highly divergent sequences like DNA dependent DNA polymerases. They constitute an excellent example, since they are very ancient genes, protein pairs share 20-30% sequence identity, and many experimental structures are available.However, protein structure comparison is not a simple task. Several computer programs are available to perform alignments and the solutions produced are often completely distinct or even defective from a biological point of view. The lack of a standard comparison methodology between alignments makes difficult to evaluate them and select a biologically relevant solution from a set of alternative alignments.Motivated by cases of difficult structural alignments observed in DNA polymerases we have implemented a new software based on dynamic programming that uses a previously computed optimal superposition of two structures to generate a new optimal alignment and to calculate structural similarity values that can be used as standard to select the best structural alignment from a set of solutions. We show several examples of structural misalignments in catalytic domains of DNA polymerases identified by our software. Finally, to conduct this class of analysis on other protein families we propose to use several of the most reliable programs in parallel and to choose the optimal alignment in the way reported here. ACKNOWLEDGEMENTS This research was funded by grants from FONDECYT (1110400), ICM (P09-016-F) and CONICYT Fellowship postgraduate studies. .

Docking and in-vitro study of human lipoxygenases inhibitors. Mascayano, C.[1], Espinosa, V.[2], Sepulveda-Boza, S.[2], Holman, T.[3]. Departamento de Ciencias del Ambiente, Facultad de Química y Biología,Universidad de Santiago de Chile[1], Escuela de Medicina, Facultad de Ciencias Médicas, Universidad de Santiago[2], Department of Chemistry and Biochemistry, University of California, Santa Cruz[3]. Sponsored by F. Gonzalez Nilo.
The lipoxygenases (LO's) are a family of non-heme iron-containing dioxygenases which catalyze the stereospecific insertion of molecular oxygen into arachidonic acid. In this research we will presents the biological evaluation of 16 compounds against 5-hLO, platelet 12-hLO, reticulocyte 15-hLO-1 and prostate epithelial 15-HLO-2, their results were compared against IC50 values and baicalein as control. The results indicated that the compounds with inhibitory activity against both enzymes (12- and 15-hLO) are also able to inhibit 5-hLO, except the compound named 3c which only inhibit 5-hLO. The size of binding site of the enzymes was related with the inhibitory action of isoflavonoids, as example the compound 1b inhibits 5-hLO with IC50 1.5 uM and 12-hLO IC50 13 uM (binding site 6% smaller than 5-hLO) and is less active with both 15-hLO isoforms (binding site 20% smaller than 5-hLO). Aditionally, the isoflavans (serie 5) with a C ring more flexible than isoflavones and baicalein were able to inhibit all tested LO's with IC50 < 40 uM.In addition, docking studies were carried out for the best and most selective inhibitors of LO's.Financial support from DICYT-USACH project # 021041MC is gratefully acknowledged.

Following cold denaturation of phosphofructokinase-2 by amide hydrogen/deuterium (H/D) exchange mass spectrometry. Ramírez, C.[1], Baez, M.[1], Wilson, C.A.M.[1], Babul, J.[1], Komives, E.[2], Guixé, V.[1]. Department of Biology, Faculty of Sciences, University of Chile, Santiago, Chile.[1], Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla CA 92093-0359, USA[2].
Phosphofructokinase-2 (Pfk-2) from *Escherichia coli* is a dimer that shows an intriguing cold denaturation process that leads to a monomeric state with low content of secondary structure, as shown by equilibrium CD, DLS and fluorescence experiments. Although equilibrium experiments with different protein concentrations have demonstrated both dissociation and unfolding of the monomers upon cold denaturation, whether both processes occur simultaneously during kinetic unfolding remains unexplored. Amide hydrogen/ deuterium (H/D) exchange mass spectrometry has been successfully applied for understanding local structural properties of proteins and to study protein-protein interactions and folding. We used this method to follow cold denaturation of Pfk-2 in deuterated water at 4 C by measuring deuteron incorporation by MALDI-TOF mass spectrometry of 7 different regions of Pfk-2 (representing both the interface and overall structure) produced after pepsin digestion. Samples taken between 0-12 hours after exposure to low temperature show similar increases in exchange across all of the regions but lower than the maximum exchange expected for unfolded proteins, thus describing a partially unfolded conformation. Fitting the maximum of deuterons incorporated for each peptide over time to a single exponential function shows that the rate of unfolding monitored by H/D exchange is, on average, $1.3x10^{-4}$ +/- $0.3x10^{-4}$ s^{-1}. A similar rate was obtained by monitoring intrinsic fluorescence changes during cold denaturation. These results suggest that cold denaturation of Pfk-2 leads to a partially unfolded monomeric state where dissociation and global unfolding occur simultaneously. Fondecyt 1090336 and 1110137.

Widen minor groove is a common feature in damaged DNA and protein-damaged-DNA complexes. Cifuentes, 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, Alameda 340, Santiago, Chile[2].
DNA damage and repair plays a central role in aging and cancer. How damage detection proteins (DDPs) can recognize different adducts embedded in the genome and how different proteins can detect the same adduct is currently unknown. Trough a comprehensive comparative analysis of the known three-dimensional structures of protein-damaged-DNA complexes and isolated damaged-DNA structures, here we show that minor groove is widened at the lesion point. This widen minor groove is present in the protein damaged-DNA complexes but also in isolated damaged DNA structures. Depending on the nature of the lesion, the opposite base to the adduct and the surrounded sequence can influence the net value of minor groove width, but ?in contrast with normal B DNA structures-in the vast majority of the lesions, minor groove is widen in the center (where the lesion is placed) and relatively more narrow in the extremes. The width of minor groove can be used as criterion to discriminate DDP complexes from other protein-DNA complexes. Surprisingly other proteins such as Tata Binding protein (TBP) and High Mobility Group B (HMGB) also satisfy this criterion. Notably, the DNA targets of those proteins have increaded minor groove widths and have been documented that they can also recognize damaged DNA. These findings suggest a common minor groove shape readout mechanism based on a widened minor groove DNA target. ACKNOWLEDGEMENTS: This research was funded by grants from FONDECYT (1110400) and ICM (P09-016-F).

Quenching of the intrinsic fluorescence of *Chlorobium tepidum* Ferritin by Iron. The role of the Ferroxidase Center in the mineralization process. Yévenes, A.[1], Espinoza, R.[2], Márquez, V.[3], Sandoval, C.[1], González-Nilo, F.[1], Lopez-Castro, J.[4], Domínguez-Vera , J.M.[5], Watt, R.K.[6]. Universidad de Talca[1], Universidad de Santiago[2], Fraunhofer Chile Research[3], Universidad de Cadiz[4], Universidad de Granada[5], Brigham Young University[6].

Ferritins (Ftn) 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 (CtFtn) is able to store iron, but the iron storage capacity and ferroxidase activity is less than that reported for Ftns from other organisms. A homology model of CtFtn shows that three Trp residues are closed to the ferroxidase center (FC), where iron are oxidized, and might be used to analyze the mechanism of iron entry into the protein. The incubation of CtFtn with iron produced a significant quenching that it is stable by 72 hours and no recovery of fluorescence was observed. These data are not consistent with a mechanism in which the FC of CtFtn acts as a pore for transfer of iron into the protein cavity as was described for another Ftns where a recovery of its fluorescence was observed. The homology model of CtFtn shows that the electrostatic properties of 3- and 4-fold channels appear to prevent the entry of iron into the inner cavity of the protein. The High Angle Annular Dark Field microscopy shows that the CtFtn present a limited store iron capacity, in agreement with the theoretic and experimental results. Supported by Proyecto Inserción en la Academia 79090038.

The 5`untranslated region of the HIV-2 mRNA harbors an internal ribosome entry site element. Letelier, A.[1], López-Lastra, M.[1]. Laboratorio de Virología Molecular, Centro de Investigaciones Médicas, Facultad de Medicina, Pontificia Universidad Católica de Chile.[1].

The human immunodeficiency virus type 2 (HIV-2) is the main etiological agent of AIDS in West Africa. For the translation initiation of viral Gag protein, the genomic RNA uses an internal ribosome entry sites (IRES) present in the Gag coding region. Unlike other lentivirus, IRES activity in the 5`untranslated region (5`UTR) has not yet been described. Recently, an HIV-2 5`UTR splice variant of the genomic RNA was reported. *In vitro* and *ex vivo* assays showed that this variant exhibits an increased translational efficiency compared with the full-length 5`UTR. These experiments were performed in a monocistronic context, which does not allow discriminating between cap-dependent and IRES-dependent translational initiation. In this study we analyzed the putative IRES-dependent translational initiation of both the full-length 5`UTR and its splice variant using bicistronic constructs. *Ex vivo* assays showed that the full-length HIV-2 5`UTR displays IRES activity, while the 5`UTR splice variant showed a decreased IRES activity compared to full-length HIV-2 5`UTR in HeLa and COS-7 cell lines. Additionally, both constructs showed inefficient translation in 293T cells. These results indicate the existence of an IRES element on the full-length HIV-2 5`UTR, and suggest that this activity is cell type-dependent. CONICYT Scholarship, FONDECYT 1090318.

Development of antibodies against iNOS of fish: A molecular marker of immune response in gill of rainbow trout. Valenzuela, C.[1], Guzmán, F.[2], Rojas, V.[1], Mercado, L.[1,2]. Grupo de Marcadores Inmunológicos en Organismos Acuáticos. Laboratorio de Genética e Inmunología Molecular (GIM), Instituto de Biología[1], Núcleo Biotecnológico Curauma (NBC). Pontificia Universidad Católica de Valparaíso, Chile[2].

TThe phagocytic activity in fish plays a key role in the clearance of different pathogens. The overproduction of reactive oxygen species (ROS) and nitrogen species (RNS) in the phagosome triggers the partial or total destruction of the pathogen. The high production of RNS such as nitric oxide (NO) in macrophages is carried out by the inducible nitric oxide synthase (iNOS) in response to pathogen-associated molecular patterns. Classically, phagocytic activity was evaluated by measuring NO, but this requires tests that exceed 72 hours which is not possible to make in tissues. In this context, the immunodetection of iNOS can be used as a direct marker of the immune response in fish. The main objective of this work was the production of monospecific antibodies against epitopes of iNOS to develop an indirect ELISA, Sandwich ELISA and immunohistochemistry for the quantification and detection of iNOS, respectively, in gill of challenged trout. CF1 strain mice were immunized intraperitoneally, at day 30 post immunization ascitic fluid rich in immunoglobulins (IgGs) anti-epitope were obtained .The IgGs were purified by affinity chromatography CNBr and its specificity was evaluated by indirect ELISA. Rainbow trout were bath challenged with the virulent structure of A. salmonicida for 24 hours. Total proteins of gills were obtained and iNOS detection was performed by indirect ELISA, Western Blot and Immunohistochemistry. In addition, iNOS expression was quantified by qRT-PCR. Furthermore, to quantify iNOS Sandwich ELISA has been standardized and is in process of validation.

Iodide (I^-) induce a rapid inhibition of Na^+/I^- symporter (NIS) through reactive oxygen species. Arriagada, A.[1], Becerra, Á.[2], Miranda, C.[1], Plaza, A.[1], Simon, F.[2], Bueno, S.[3], Kalergis, A.[3], Carrasco, N.[4], Riedel, C.[1]. Laboratorio de Biología Celular y Farmacología. Departamento de Ciencias Biológicas. Universidad Andrés Bello.[1], Laboratorio de Fisiopatología Celular y Molecular, Departamento de Ciencias Biológicas, Univerisdad Andrés Bello.[2], Millennium Institute on Immunology and Immunotherapy. Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas. Pontificia Universidad Católica de Chile.[3], Department of Molecular Pharmacology, Albert Einstein College of Medicine, USA.[4].

Iodide (I^-) uptake in the thyroid, is mediated by Na^+/I^- symporter (NIS). Elevated concentrations of I^- induces an acute inhibition of I^- uptake. The cellular mechanism that underlay this regulation is poorly understood. In this study we evaluated the effect of high levels of I^- on rapid inhibition of NIS function in vivo and in vitro, its expression and localization in thyroid cells. We also evaluated the role of reactive oxygen species (ROS) on this inhibition mediated by high I^-.Rats received high I^- diet and FRTL-5 cells, were incubated with 100 uM of KI. Under this treatment, I^- uptake assays were performed. NIS was evaluated by western blot and immunofluorescence. Rats showed a reduced I^- uptake and NIS content with high I^- diet. Immunofluorescence in thyroid slices, showed that NIS localized on the basolateral region, with no differences with the control group. FRTL-5 cells, significantly reduced the I^- uptake when incubated 2 hours with KI. However, the total amount of NIS and its presence at plasma membrane, is not modified. This uptake inhibition, is accompained by a significant increase in ROS. Pharmacological inhibitors of ROS, recovered I^- uptake in the presence of KI.These results suggest that high I^- induce inhibition of the I^- uptake without affecting the NIS localization at plasma membrane. The excess of I^- increased ROS and these molecules are responsible for the mechanism of NIS inhibition.Grants: UNAB DI 06-08; Fondecyt # 1100926; Milenio P07/088-F .

PROTEIN STRUCTURE AND FUNCTION

Nucleotide selectivity mechanism of the ADP-dependent glucokinase from *Thermococcus litoralis*. **Merino, F.**[1], Guixé, V.[1]. Laboratorio de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Chile[1].
Some archaea of the Euryarchaeota use a modified version of the Embden-Meyerhof pathway to degrade glucose. One of the most Interesting modifications seen in this pathway is that the phosphorylations of fructose-6-phosphate and glucose are catalyzed by two homologous enzymes that use ADP and not ATP as the phosphoryl source. This modification of the glycolysis has been misleadingly attributed to thermophilic life, but its real adaptive role is still not well understood. Moreover, the structural determinants of the nucleotide discrimination have not been explored. While these enzymes do not show significant sequence identity with any known ATP dependent kinase they can be structurally classified as members of the ribokinase superfamily. In this work, we have studied the nucleotide specificity of the ADP-dependent glucokinase from *Thermococcus litoralis* by means of isothermal titration calorimetry as well as molecular dynamics simulations. The results show that while ATP cannot act as a phosphoryl donor it still can bind to the enzyme. Moreover, while MgADP is the true substrate of this enzyme, free ADP can also bind to the enzyme with an affinity similar to that of ATP. Then, in order to understand the role of the magnesium ion in the modulation of the nucleotide affinity we performed molecular dynamics simulations. Surprisingly, the simulations show that a couple of arginine residues act as an electrostatic trap where any molecule with a total charge higher than -1 is pulled out from the binding site. Fondecyt 1110137.

Independence between thermal and mechanical stability in a hyperthermophilic enzyme. Single molecule studies reveal the modulation of the mechanical stability triggered by ligands. **Rivas-Pardo, J.A.**[1], Alegre-Cebollada, J.[2], Ramírez, C.[1], Fernández, J.M.[2], Guixé, V.[1]. Facultad de Ciencias, Universidad de Chile, Santiago, Chile[1], Department of Biological Sciences, Columbia University, New York, USA[2].
The ADP-dependent glucokinase from *Thermococcus litoralis* (TIGK) catalyzes the first reaction of a modified version of the Embden-Meyerhof (EM) metabolic pathway, where glucose and fructose-6-phosphate are phosphorylated by kinases that, differently to common of kinases, use ADP instead of ATP as the phosphoryl donor. TIGK shows extraordinary thermophilic properties which sustain its function at very high temperatures, close to 90 degree Celsius. Indeed, so far it has not been possible to unfold TIGK using either temperature or chemical denaturants. We have previously demonstrated that the enzyme has different conformational arrangements in solution depending on whether the substrates are bound to the active site. The enzyme without ligands is in an open conformation, whereas with the substrates, the enzyme shows domain closure. We proposed that these different conformational arrangements are a consequence of an induced fit mechanism. In this work we studied the mechanical unfolding of TIGK by single-molecule force spectroscopy. We found that the protein is mechanically labile, despite its great stability at high temperatures. Thus, for the first time, we have been able to unfold an otherwise highly stable protein. The binding of either ADP or ADP and glucose increase the mechanical stability of TIGK in agreement with the proposed catalytic mechanism. The strategy employed in this work may be an appropriate tool to investigate sequential binding mechanisms in enzymes. (Fondecyt 1110137).

The role of Heme oxygenase 1 in Systemic Lupus Erithematosus. **Mackern, J.P.**[1], Herrada, A.[2], Carreño, L.J.[3], Gómez, R.S.[2], Anegón, I.[4], Jacobelli, S.H.[5], Llanos, C.[5], Kalergis, A.M.[2,5,6]. 1Millennium Institute on Immunology and Immunotherapy. Departamento de Genética Molecular. Facultad de Ciencias Biológicas. Pontificia Universidad Católica de Chile, Santiago, Chile.[1], Millennium Institute on Immunology and Immunotherapy. Departamento de Genética Molecular. Facultad de Ciencias Biológicas. Pontificia Universidad Católica de Chile, Santiago, Chile.[2], Millennium Institute on Immunology and Immunotherapy. Departamento de Genética Molecular. Facultad de Ciencias Biológicas. Pontificia Universidad Católica de Chile, Santiago, Chile[3], INSERM UMR 643, CHU de Nantes. France.[4], Departamento de Reumatología, Facultad de Medicina. Pontificia Universidad Católica de Chile, Santiago, Chile.[5], [6].
Heme oxygenase (HO) is an enzyme that catalyzes heme. The HO-1 is an inducible isoform with immunoregulatory functions. HO-1 induction is associated with immune regulation as suppression of Th1- cytokine production. The aim of this work was to study HO-1 levels in Systemic Lupus Erithematosus (SLE) patients and evaluate the beneficial effects of an HO-1 inducer in a mice model of SLE, C57BL/6 Fc?RIIb KO. PBMCs were obtained from 20 SLE patients and 10 Healthy controls (HC). Expression of HO-1, MHC class II and CD86 was measured by flow cytometry. 17 weeks old Fc?RIIb KO were used (n=9), 5 mice were inoculated with Cobalt-Protoporphyrin (CoPP)(ip) for 6 months. A hallmark of SLE is nephritis, which could be assessed by proteinuria reactive strips. In this study, we show that CD14+ cells from SLE patients expressed less HO-1 than did cells of HC (relative expression, HC = 1.03?0.08 and LES = 0.67?0.04). HO-1 expression was not changed in CD4+ and CD11c+ cells from SLE patients. The expression of MHCII and CD86 on CD14+ cells from SLE patients were similar to HC. When the HO-1 inducer CoPP was chronically administered to Fc?RIIb KO mice we observed a diminished incidence (0%) of proteinuria as compared to control mice (50%). Reduced expression of HO-1 in monocytes from SLE patients suggests that HO-1 may be involved in SLE pathology. This notion is supported by the prevention of glomerulonephritis in SLE-prone mice by the induction of HO-1.

Expression and biochemical analysis of AtSDL, a putative sorbitol dehydrogenase in *Arabidopsis thaliana*. **Aguayo, F.**[1], Ampuero, D.[1], Parada, R.[1], Mandujano, P.[1], Handford, M.[1]. Laboratorio de Biología Molecular Vegetal, Departamento de Biología, Facultad de Ciencias, Universidad de Chile.[1].
Sugar alcohols, including sorbitol, mannitol and xylitol, perform a variety of roles in plants. The metabolism of sorbitol is well-characterised in *Rosaceae* species where this polyol is the major phloem-translocated carbohydrate. In these species, sorbitol is catabolised to fructose in sink organs by sorbitol dehydrogenase (SDH). However, in almost all other plant families, sucrose is the principal phloem-transported sugar and polyols are present in low basal levels. Sorbitol and/or SDH activity have been detected in some of these species, although their physiological roles have yet to be understood. As an approach to determine the roles of SDH in non-sorbitol translocating species, we have identified a protein in *Arabidopsis thaliana* with >75% amino acid identity with previously-characterised plant SDHs, named AtSDL. Molecular analysis showed that AtSDL is a cytosolic protein. *AtSDL* is expressed in multiple organs and analysis of Arabidopsis which had been stably-transformed with an *AtSDL* promoter::GUS fusion construct, revealed almost systemic expression. Analysis *in silico* using three-dimensional modelling and molecular docking studies of the protein indicate that NAD+ and zinc are acceptable cofactors and that multiple polyols are capable of interacting with the enzyme. In order to corroborate these results experimentally, biochemical analysis using *in vitro* translation, yeast and bacterial expression systems was performed, revealing that recombinant AtSDL possesses the ability to oxidise preferentially sorbitol and xylitol among the polyols found in Arabidopsis in the presence of both cofactors. Funding: Fondecyt 1100129, CONICYT Magister 22110701 (Aguayo) and 22100522 (Ampuero).

Interactions defining the strong specificity for NADP in *Escherichia coli* glucose-6-P dehydrogenase. Cabrera, R.[1], Fuentealba, M.[2], Muñoz, R.[3]. Departamento de Biología, Facultad de Ciencias, Universidad de Chile.[1], Universidad de Chile[2], Universidad Andres Bello[3].

In heterotrophic microorganisms, NADH and NADPH are produced by the dehydrogenasesfrom the central pathways. Although differing only by the presence of a phosphate group atthe adenosine moiety, they are differentially and massively consumed by cellular process suchas respiration (NADH) and biosynthesis (NADPH). In *Escherichia coli*, the oxidative branch ofthe pentose-phosphate pathway might contribute with more than half of the whole NADPHproduction when glucose is used as the sole carbon source. In this work, we explore throughsite-directed mutagenesis and molecular simulations how the highly specific NADP-dependentglucose-6-P dehydrogenase from *Escherichia coli* (EcG6PDH) discriminates against NAD. Sequencealignment of bacterial G6PDHs indicates that the strong preference for NADP correlates withthe presence of two positive residues in non-conserved positions that could interact with the 2'-phosphate of NADP. A battery of single and double mutants of Lys18 and Arg50 in EcG6PDH showthat the loss of both positive charges is needed to completely abolish the preference for NADP. The replacement for residues with the ability to form hydrogen bonds is not enough to increaseNAD affinity. Molecular simulations using a homology model of EcG6PDH in complex with glucose-6-P and either NADP or NAD, show a crucial interaction of the diphosphate moiety with Arg222 that is lost when the phosphate group is absent from the cofactor. We propose how the specificityof the EcG6PDH could be reverted to high affinity towards NAD and discrimination against NADP.FONDECYT 11080290.

Effect of citrate on the activity of mammalian Fructose 1,6-bisphosphatase. Asenjo, J.L.[1], Díaz, A.[2], Maureira, M.A.[1], Schott , S.[1], Yáñez, A.J.[1], Guinovart, J.J.[2], Siebe, J.C.[1]. Instituto de Bioquímica y Microbiología, Universidad Austral de Chile, Valdivia[1], Institut de Recer?a Biomédica, Barcelona, Spain.[2].

To meet metabolic needs and ensure that futile cycling does not occur under normal circumstances, gluconeogenesis and glycolysis are regulated coordinately and reciprocally. A control point in gluconeogenesis is the reaction catalyzed by the Mg^{2+}-dependent fructose 1,6-bisphosphatase (FBPase), which is strongly inhibited by AMP, fructose-2,6-bisphosphate (F-2,6-P_2) and high substrate concentrations (F-1,6-P_2 >30uM). Citrate, is also an allosteric regulator of several glycolytic and gluconeogenic enzymes. The effect of citrate on FBPase activity is still controversial. It has been postulated that citrate activates E. coli FBPase while inhibits FBPase from grapefruit. This knowledge and knowing that citrate is an inhibitor of phosphosfructokinase, prompted us to examine the effect of citrate on mammalian FBPase. Increase in citrate concentration showed that FBPase activity decreased, probably due to Mg^{2+} chelation. The enzyme activity as function of free citrate concentrations at fixed variable free magnesium concentrations provoked a dose-dependent increase on FBPase activity only at low free Mg^{2+} concentrations (? 0,5 mM). Fluorescence studies using an FBPase mutant (Phe16Trp) proved that citrate binds to the enzyme. A 3-D computer model confirmed that citrate binds at the tetramer C1-C4 interface. These results suggest that citrate has a dual effect: 1) stabilization of an active tetramer; 2) modulation of gluconeogenic oscillations generated by repeated bursts of FBPase activity involving Mg^{2+} concentration. The results raise the possibility that FBPase activity oscillates in vivo in response to the levels of allosteric effectors and substrates. (FONDECYT: 1090740; DID-UACh).

Structural-functional determinants of the intracellular ascorbic acid binding domain in SVCT1. Haensgen, H.[1], Salas-Burgos, A.[1], Sepulveda, M.A.[1], Rivas, C.[1], Vera, J.C.[1]. Departamento de Fisiopatología, Facultad de Ciencias Biológicas, Universidad de Concepción. Concepción, Chile.[1].

SVCT1 is a sodium-coupled ascorbic acid transporter that is functionally characterized, but we lack information on structural elements involved in interactions of ascorbic acid with the protein. SVCT1 belongs to the Mayor Facilitative Superfamily (MFS), and therefore we used Lactose Permease 3D-crystals as model to identify, by structural homology, amino acid residues involved in the intracellular ascorbic acid binding domain in SVCT1. These amino acid residues where individually replaced by site-directed mutagenesis, and the mutant proteins were expressed in HEK-293 cells followed by determining their subcellular localization by confocal microscopy and characterizing their functional properties by ascorbic acid transport experiments. Mutant proteins were succesfully sorted to the plasma membrane, however, there were important variations in functional parameters like markedly decreased ascorbic acid transport activity, major increase in the ascorbic acid transport Km and alterations in sensibility to sodium. Recently, a refined phylogenetic analysis revealed that SVCT1 doesn't belong to the MFS but instead belongs to the NAT family. The crystallization of the uracil transporter allowed us to generate a novel SVCT1 3D-model that allowed us to explain in a more robust manner the changes observed in the functional properties of the mutant proteins. Overall, our data support the concept that there is a rather restricted number of possible arrengements of the transmembrane domains in transporters belonging to different families defined by structural-functional differences, and highlight the importance of structural homology versus sequence conservation. Beca CONICYT-Tesis 24090152,Beca MECESUP UCO0606, Proyecto FONDECYT 1090501.

Characterization of *Daucus carota* lycopene b-cyclase gene (*lcyb1*) by over-expression in plant models. Moreno, J.C.[1], Stange, C.[1]. Facultad de Ciencias, Universidad de Chile[1].

Carotenoids are isoprenoid pigments involved in photosynthesis, photo-protection and hormone synthesis in plants. They are produced in plastids and one of the key regulatory steps of the biosynthesis is mediated by the lycopene b-cyclase enzyme (LCYB) that cycles lycopene to give rise to the orange pigment, b-carotene, a vitamin A precursor with strong antioxidant properties.In carrot (*Daucus carota*) two *lcyb* genes (*lcyb1* and *lcyb2*) have been reported. *Dclcyb1* expression presents the highest increase throughout the plant development and post-transcriptional gene silencing of *Dclcyb1* decreases total carotenoid levels. In addition, through heterologous complementation in *E. coli*, we prove that *Dclcyb1* gene codifies for a functional enzyme. Here, we determined that DcLCYB1 presents a plastid localyzation, by means of transiently transformation in *N. tabacum* and we over expressed the gene in tobacco and carrot in order to complement the characterization of *Dclcyb1*, and to verify its function in planta. *Dclcyb1* expression increases between 12 to 300 fold in transgenic tobaccos and between 0,7 and 9 fold in carrot lines. Transgenic tobaccos also express the exogenous DcLCYB1 protein, determined through western blot. Almost all tobacco and carrot lines exert an increase in total carotenoids, lutein, chlorophyll and b-carotene. Taken together, *Dclcyb1* gene is functional in plants and can be applied in metabolic enginnering of plants with high comercial value. Aknowledgement to Fondecyt 11080066.

MOLECULAR BIOLOGY OF THE CELL I

Evaluation of antitumor therapy in a murine model of bladder cancer. Rivas, A.[1,2,3], Borgna, V.[1,2,3], Landerer, E.[1,3], Avila, M.[1,3], Burzio, V.[1,2,3], Villegas, J.[1,2,3]. Andes Biotechnologies S.A.[1], Fundación ciencia para la vida[2], Universidad Andres Bello[3].

Bladder cancer is the second most common cancer of the genitourinary tract and so far no highly effective therapy exists against this disease. In our laboratory, we have described a family of RNA molecules of mitochondrial origin, where the knock-down of one of its members, the Antisense non-coding mitochondrial RNA (ASncmtRNA), triggers a massive degree of cell death by apoptosis. This work aims to assess the application of this property to antitumor therapy in a mouse model of bladder cancer in vitro and in vivo. For this purpose, assays were performed in the mouse bladder cancer cell line MB49, where cell death was induced by knock-down of the target molecule in different regions, using chemically modified antisense oligonucleotides (ASOs). Treatment was assessed at different time intervals and with different transfection agents. The in vivo therapeutic assays were performed in C57BL/6 mice, with different numbers of MB49 cells implanted subcutaneously and after 10 days ASOs were injected every 4 days for 20 days. The treatment was assessed by measuring the decrease of tumor growth by calculating the volume of palpable tumor and the survival of the animal. The results are encouraging, but the development of this new therapy necessarily requires the use of an orthotopic model and a model of human cells in nude mice before entering a preclinical phase. Grants FODECYT 1110835, UNAB DI-20-11-I.

Draft genome assembly of *Penicillium purpurogenum* using next-generation sequencing techniques and identification of possible lignocellulolytic enzyme genes. Mardones, W.[1], Eyzaguirre, J.[1]. Universidad Andrés Bello[1].

The filamentous fungus *P. purpurogenum* grows on a variety of agro-industrial wastes and efficiently degrades lignocellulose (main component of plant cell wall). Among the substrates are wheat straw, rich in xylan and cellulose, and sugar-beet pulp, with high content in pectin and cellulose. For this purpose, the fungus secretes a number of cellulolytic, xylanolytic and pectinolytic enzymes. The genome sequence of *P. purpurogenum* is not available; this hinders the search for possible lignocellulolytic genes. However, next-generation sequencing (NGS) technologies currently allow whole genome sequencing at a low cost, and in conjunction with the development of new software for genome assembly, generate high quality draft genome sequences. In this work we have obtained a draft genome sequence of *P. purpurogenum* using Solexa / Illumina as sequencing technology. The draft genome was assembled using a phylogenetically close fungus (*Aspergillus fumigatus* af293) as reference genome. We have identified a large number of genes, some of which may be involved in lignocellulose degradation. This is the first draft genome of *P. purpurogenum* and represents a great step forward in the study of this organism. Support: FONDECYT 1100084 and UNAB DI-03-10/R.

Toxin-antitoxin systems in a mobile genetic element from *Acidithiobacillus ferrooxidans*. Bustamante, P.[1], Orellana, O.[1]. Programa de Biología Celular y Molecular, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile[1].

Bacterial toxin-antitoxin (TA) systems consist of pairs of genes encoding for a stable toxin that disrupts essential cellular process and a labile antitoxin that binds to and blocks the activity of the toxin. Plasmid-encoded TA systems participate in plasmid stabilization by a mechanism denoted as post-segregational killing. However, the role of chromosomal TA systems in the cell physiology is enigmatic. Proposed functions are gene regulation, programmed cell death and response to general stress. Chromosomal TA systems are also believed to participate in the maintenance of mobile genetic elements. *Acidithiobacillus ferrooxidans* ATCC 23270, an acidophilic, chemolithoautotrophic Gram-negative gamma-proteobacterium, contains in its genome a 300 kb active integrative-conjugative element, designated ICE*Afe*1. This mobile genetic element encodes some putative TA systems. In this study, we show that these putative genes are organized as typical TA systems and are transcriptionally active. We carry out the characterization of the ortholog of MazEF system (E: antitoxin, F: toxin) encoded in the ICE*Afe*1. *MazE* and *mazF* are co-transcribed in *A. ferrooxidans*. When the genes are co-expressed in *E. coli*, MazE and MazF form a complex that co-purifies by affinity chromatography. MazF is an RNase which leads to cell growth arrest upon over-expression in *E. coli*. This toxicity can be reversed by co-expression of MazE. Thus, MazEF is an active TA system that is likely involved in the maintenance of ICE*Afe*1. Fondecyt 1070437 and 1110203 (OO), Beca de Apoyo AT-24100112 and Beca Conicyt (PB).

Human metapneumovirus impairs the capacity of dendritic cells to prime antigen-specific naïve T cells. Céspedes, P.F.[1,2], González , P.A.[1,2], Kalergis , A.M.[1,3]. Millennium Institute on Immunology and Immunotherapy.Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile.[1], These authors contributed equally.[2], Departamento de Reumatología. Facultad de Medicina. Pontificia Universidad Católica de Chile.[3].

Human metapneumovirus (hMPV) is a respiratory virus that mainly targets infants, the immunocompromised and the elderly. It has been estimated that hMPV is the second cause of upper and lower acute respiratory tract infection in infants worldwide, causing significant economic burden. Similar to other respiratory viruses, hMPV can repeatedly re-infect the host without major antigenic changes. These observations suggest that hMPV may have evolved molecular mechanisms to interfere with the onset of an adequate host immune response, preventing the establishment of a long-lasting immunological memory. Recent in vitro and in vivo studies have suggested that hMPV infects dendritic cells (DCs) and decreases their capacity to activate T cells. However, the mechanisms underlying this reduced T cell activation remain elusive. Here we show that hMPV-infected murine DCs mature poorly in response to hMPV and are rendered inefficient at activating antigen-specific naïve $CD4^+$ T cells. Decreased T cell activation was partially mediated by soluble factors secreted by hMPV-infected DCs and was virus strain-dependent. However, our results suggest inhibition of T cell activation by hMPV-infected DCs is mainly mediated by a cell contact-dependent mechanism. Taken together, our data suggest that hMPV interferes with DC function by promoting the expression of soluble and membrane-bound determinants that affect T cell activation. As a result, it is likely that hMPV can negatively modulate the anti-viral adaptive immunity in susceptible hosts.Work supported by FONDECYT Grants 3100090, 1070352, 1110397 and Millennium Institute on Immunology and Immunotherapy (P07/088-F).

Caveolin-1 phosphorylation on tyrosine-14 sensitizes b-pancreatic Min-6 cells to palmitate-induced apoptosis. Núñez, S.[1], Mears, D.[2], Leyton, L.[1], Quest, A.[1]. Centro Fondap de Estudios Moleculares de la Célula (CEMC), Facultad de Medicina, Universidad de Chile[1], USU School of Medicine, Bethesda, Maryland, USA.[2].
Introduction. Fatty acid induced cytotoxicity is thought to be an important factor to b-pancreatic cell death in Type II diabetes; however, the underlying mechanisms remain unclear. Caveolin-1 is normally expressed in b-pancreatic cells. Previously, we demonstrated that Caveolin-1 presence enhances apoptosis induced by palmitate in a b-pancreatic cell line. Here, we investigated the molecular mechanisms by which Caveolin-1 sensitizes these cells to palmitate-induced apoptosis. Material and methods. Min-6, a mouse b-cell line lacking Caveolin-1, was stably transfected with either empty vector or Caveolin-1-encoding plasmid and then incubated with palmitate or C2-ceramide. Cell viability and generation of reactive oxygen species (ROS) was measured by flow-cytometry. Phosphorylation of Caveolin-1 on tyrosine-14 was evaluated by Western blotting. Results. Both palmitate and C2-ceramide treatment induced ROS formation that was inhibited by myriocin, a ceramide synthesis inhibitor. As with palmitate, the presence of Caveolin-1 enhanced apoptosis induced by C2-ceramide. N-acetylcysteine protected against palmitate and C2-ceramide-induced cell death. Palmitate-induced phosphorylation of Caveolin-1 on tyrosine-14 was inhibited by the Src family inhibitor PP2, which also reduced Caveolin-1-enhanced cell death in response to palmitate. Conclusion. Palmitate induced ROS in a manner dependent on the synthesis of ceramide. Addition of C2-ceramide mimics the effects of palmitate. Caveolin-1 presence did not alter ROS formation; instead, Caveolin-1 phosphorylation increased in the presence of palmitate. Inhibition of this phosphorylation event reduced Caveolin-1-mediated sensitization. Thus, Caveolin-1 sensitization of Min-6 to palmitate-induced b-cell death is the consequence of ROS-dependent phosphorylation on tyrosine-14.

Proinflammatory cytokines as potential immunological markers in rainbow trout (*Oncorhynchuss mykiss*) larvae. Santana, P.[1], Guzmán, F.[2], Mercado, L.[1,2]. Grupo de Marcadores Inmunológicos en Organismos Acuáticos. Laboratorio de Genética e Inmunología Molecular (GIM), Instituto de Biología. [1]. Núcleo Biotecnológico Curauma (NBC). Pontificia Universidad Católica de Valparaíso. Chile.[2].
The larval stage of salmonids is affected by high mortality, which is largely due to opportunistic pathogens such as IPN virus, bacteria of the genus Vibrio, Flavobacterium, Aeromonas and the fungus Saprolegnia sp.. These together with factors such as high fish densities and poor water quality, weakens their immunity causing diseases, low production and finally, economic losses. To prevent and control diseases the industry has invested heavily in the use of immunoprophylaxis such as vaccination and immunostimulation. However, to evaluate the different strategies used is necessary to know and understand the ontogeny of the immune response capacity in larvae. The aim of this work is to evaluate the ability of the immune response of larvae through immunological markers at protein level. In earlier stages transcriptional activity of IL-8, IL-8R, TNF-a and IL-1B have been detected in some larvae, different from salmonids. For the above, we used monospecific polyclonal antibodies against these molecules and quantification of their expression by ELISA and immunohistochemistry in 10 to 33 days post-eclosion rainbow trout larvae, stimulated and non-stimulated with LPS from Pseudomonas aeuruginosa. Preliminary analysis has shown the capacity of larval immune response when challenged was applied, which is reflected in the expression of these cytokines at skin, gills, gut and kidney level. These results are the basis to develop a Sandwich ELISA to quantify biomarkers, with potential applications to the salmon industry. Becaria CONICYT.

IgG prevents subversion of dendritic cell phagocytosis by virulent *Salmonella* and restores antigen presentation to T cells. Riquelme, S.[1], Bueno, S.[1], Kalergis, A.[1,2]. Millennium Institute of Immunology and Immunotherapy. Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas. Pontificia Universidad Católica de Chile.[1], Departamento de Reumatología, Facultad de Medicina. Pontificia Universidad Católica de Chile.[2].
Dendritic cells (DCs) are professional antigen presenting cells that can prime naïve T cells and initiate anti-bacterial immunity. However, the bacteria *Salmonella typhimurium* can prevent DCs from activating antigen-specific T cells by two mechanisms: reduction of phagocytosis in a PI3K and *Salmonella Pathogenicity Island 1* (SPI-1)-dependent manner and reduction of bacterial-derived antigens presentation to T cells. Recent studies have shown that IgG-opsonization of *Salmonella* can restore the capacity of DCs to present pMHC complexes to T cells. Interestingly, T cell activation requires Fc?RIII expression over DCs surface, suggesting that this receptor could counteract both antigen presentation and phagocytosis evasion by bacteria. Here we show that, despite IgG-coated *Salmonella* retains its capacity to secrete anti-capture SPI-1 proteins, DCs are efficiently capable to engulf high load of IgG-coated bacteria. These results suggest that DCs employ a mechanism to engulf IgG-coated *Salmonella* that is different from that used for free bacteria. In this context, we noted that DCs do not employ PI3K or the actin cytoskeleton to capture IgG-coated bacteria. Likewise, we observed that the capture is an Fc?RIII-independent mechanism. Interestingly, these internalized bacteria were rapidly targeted for degradation within lysosomal compartments. Thus, our results suggest a novel mechanism in DCs that does not employ PI3K/ actin cytoskeleton/Fc?RIII to engulf IgG-coated *Salmonella*, is not affected by anti-capture SPI-1-derived effectors and enhance DC immunogenicity, bacterial degradation and antigen presentation.This work was supported by FONDECYT 1085281, 1070352, 3070018, Millennium Nucleus on Immunology and Immunotherapy P04/030-F.

Development of polyclonal antibody anti-Vtg and an enzyme-linked immunosorbent assay ELISA for vitellogenin measurement in Paralichthys adspersus used in endocrine disruptor screening. Leonardi, M.[1,2], Bustos , P.[3], Puchi, M.[4], Romo, X.[1], Morin, V.[4]. 1. Depto de Ciencias Biologicas, Universidad Andres Bello[1], Programa de Investigacion Marina de Excelencia Universidad de Concepcion[2], Depto de Bioquimica Clinica e Inmunologia, Universidad de Concepcion[3], Depto de Bioquímica y Biologia Molecular, Universidad de Concepcion[4].
The yolk protein precursor vitellogenin (Vtg) in plasma has proved to be a simple and sensitive biomarker for assessing exposure of fish to environmental estrogens. In this study we have developed polyclonal antibody anti-Vtg and quantitative enzyme linked immunosorbent assays (ELISAs) for Vtg in Paralichthys adspersus, main sentinel species in studies designed to monitor marine environmental quality off the Chilean coast. Polyclonal antibodies were obtained one against a synthetic peptide corresponding to an immunogenic sequence of the Vg from Chilean flounder. A titer of 1:4000 was achieved for the anti-Vtg immunoglobulin G antibodies as determined by enzyme-linked immunosorbent assay. Specific reactivity against Chilean flounder Vtg was assessed by immunoblot, and antibodies did not have not cross-reactivity with plasma proteins of male Chilean flounder. A competitive, antibody-capture, quantitative ELISA was then developed based on the purified Vtg and policlonal antibody. The ELISA was validated by demonstrating parallel binding slopes of dilution curves prepared with plasma from E2-injected males and vitellogenic females compared with purified Vg standard. High levels of plasmatic Vg from male flounders caught off the Bio-Bio region coastline in central Chile were determinate, registering mean valor of 279.77ng/ml, up to 2280ng/ml. 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 in the area studied. Grant: PIMEX Nueva-Aldea Program.

MOLECULAR BIOLOGY OF THE CELL II

HsUGTrel1 is a human UDP-glucose transporter with three splicing variants that are upregulated by the unfolded protein response. Donoso, M.[1], Moreno, I.[1], Temple, H.[1], Moreno, A.[1], **Orellana, A.**[1]. FONDAP Centro de Regulación del Genoma, Núcleo Milenio en Biotecnología Celular Vegetal,Centro de Biotecnología Vegetal, Facultad de Ciencias Biológicas, Universidad Andrés Bello[1].

UDP-glucose is a key molecule in the N-glycoproteins quality control process within the endoplasmic reticulum (ER). This nucleotide sugar is synthesized in the cytosol and we have found that is incorporated into the ER by the nucleotide-sugar transporter (NST) HsUGTrel1. We found three splicing variants of HsUGTrel1 in human cells (HsUGTrel1-A, -B and -C). The present work addresses the function of the HsUGTrel1 splicing variants and its role in the incorporation of UDP glucose into the ER lumen.We performed in vitro transport assays using reconstituted liposomes and found that HsUGTrel1A and HsUGTrel1C are able to transport UDP-glucose but no other nucleotide sugar tested. Subcelullar localization was determined using splicing variants fused to GFP and the results show that HsUGTrel1 A is localized in the ER, ERGIC and cis-Golgi in HeLa cells, while the other two splicing variants are localized only in the ER. Using qRT-PCR we found that HsUGTrel1 splicing variants are differentially accumulated under the activation of unfolded protein response (UPR). Our data support the role of HsUGTrel1A and C in the transport of UDP-glucose inside the ER lumen. Supported by FONDAP CRG-15090007; PCB-MN P02-009F; Fondecyt 1070379. AM and IM are supported by Programa de Formación de Capital Humano Avanzado CONICYT.

Molecular effect of lithium on Sertoli cells. Maldonado, R.[1], Villarroel-Espíndola, F.[1], Torres, C.[1], Cereceda, K.[1], Van der Stelt, K.[1], Lopez, C.[1], Salazar, E.[1], Covarrubias, A.[1], Angulo, C.[1], Castro, M.[1], Slebe, J.C.[1], Concha, I.I.[1]. Instituto de Bioquímica y Microbiología, Universidad Austral de Chile[1].

Lithium is used as a mood stabilizer drug during bipolar disorder treatments. Secondary effects of these treatments affect several organs, where the testis is one of the most affected. At the molecular level, this ion inhibits glycogen synthase quinase 3 beta, which regulates glycogen synthase (GS), enzyme responsible of glycogen synthesis. The aim of this study was to evaluate lithium action on seminiferous tubule, specifically Sertoli cells. Contrary to what was described in muscular and hepatic cells, the muscular isoform of GS (MGS) of these cells was insensitive to lithium indirect stimulation. In addition, this treatment produced a relocalization of Ser640 phosphorylated MGS in cytosolic granules. Electron microscopic analysis of these granules showed that they are surrounded by a membrane, which was corroborated by protease protection assays. Nevertheless, by fluorescence microscopy, we discarded the presence of autophagosomes marker proteins (LC3 and ATG12) in pSer640MGS granules. These granules only colocalized with RNA processing bodies marker proteins like Ago-2 and Staufen, as well as others involved in MGS regulation. The study of the molecular effects of lithium will help to establish the possible causes of the injuries produced on Sertoli cells and seminiferous tubule. (FONDECYT-1110508, 1110571, DID-UACh D-2011-09, Beca CONICYT-RM).

Knock-down of the Sense non-coding mitochondrial RNA (SncmtRNA) in normal cells line induces modulation in the cell cycle. Vidaurre, S.[1], Oliveira-Cruz, L.[2], Fitzpatrick, C.[1], Villegas, J.[1], Burzio , V.[1], Burzio , L.[1]. Andes Biotechnologies, Fundación ciencia para la vida, UNAB[1], Andes Biotechnologies, Fundación ciencia para la vida[2].

The noncoding mitochondrial RNAs (ncmtRNAs) display differential expression according to proliferative status. In cultured cells and tissue samples from human and mouse, normal proliferating cells express the sense (SncmtRNA) and antisense ncmtRNAs (ASncmtRNA), tumor cells express the S and down-regulate the ASncmtRNAs, while normal resting cells express neither (Villegas et al, 2007; Burzio VA et al, 2009). Treatment with chemically modified oligonucleotides (ODNs) directed against the ASncmtRNAs induces massive and selective death of tumor cells, mediated by autophagy and apoptosis. At early post-transfection times, tumor cells show decreased proliferation and changes in levels of key cell cycle proteins. Viability of normal proliferating cells such as human keratinocytes (HFK) and melanocytes (NHEM) and mouse myoblasts (C2C12) is unaffected by knock-down of ASncmtRNAs. However, several data suggest an association between expression of the ncmtRNAs and cell cycle regulation. When normal human resting lymphocytes are stimulated with phytohaemagglutinin to enter S-phase, both S and AS transcripts are expressed (Villegas et al, 2007). Rhodamine 6G treatment reversibly blocks expression of both transcripts, as well as DNA synthesis and PCNA expression (Burzio VA et al, 2009). Transfection of C2C12 cells with an ODN targeting the SncmtRNA results in G1 arrest and decreased expression of cyclinD1 and PCNA in a reversible manner. Taken together, our results indicate a tight relationship between the ncmtRNAs and regulation of cell cycle progression and suggest a hypothetical role for SncmtRNA as an oncogene. Grants PBF-16 and FONDECYT 1110835.

AtDFG10-2 plays a role in N-glycosylation, endomembrane trafficking and development in *Arabidopsis thaliana*. Rubilar, C.[1,2], Norambuena, L.[1,2]. Laboratorio de Biología Molecular Vegetal, Facultad de Ciencias, Universidad de Chile.[1], Núcleo Milenio en Biotecnología Celular Vegetal.[2].

Endomembrane system (ES) is involved in several essential cellular processes, which are highly conserved among different eukaryotic cells. We have used chemical biology as a strategy for studying novel molecular players of the ES. The ES-perturbing drug Sortin2 has led to interesting genes related to ES functioning. One of them is the Sacharomyces cerevisiae gen scdfg10 and its Arabidopsis thaliana orthologs atdfg10-1, -2 and -3. All of the gene products share the transmembrane domain distribution and the functional domain 5-a-steroid reductase at their C-terminal. Interestingly, this domain has been related to protein N-glycosylation in humans and yeast. The Arabidopsis knockout atdfg10-2 has several body structure alterations suggesting an important role of atdfg10-2 in plant development. At cellular level this mutant has altered vacuole morphology however endocytic trafficking seems to be unaffected. To study the molecular function of atdfg10-2 we have used Sacharomyces as heterologous system. atdfg10-2 is able to complement the N-glycosylation defect of scdfg10. Additionally, the scdfg10 deletion mutant is more resistant than wild type to Sortin2 effect on vacuolar trafficking. The expression of atdfg10-2 is able to complement the Sortin2 resistant phenotype of scdfg10. However atdfg10-1 does not rescue any of the mutant phenotypes. These results suggest atdfg10-2 shares with scdfg10 the molecular and cellular function related to protein N-glycosylation as well Sortin2-action-mode within ES. atdfg10-2 function has strong impact in ES and plant development opening further studies related to N-glycosylation role in these processes. Fundings: FONDECYT11080240, ICMP06-065-F.

Differential regulation of endoplasmic reticulum-mitochondria coupling by mTOR inhibition and tunicamycin-induced endoplasmic reticulum stress. Bravo, R.[1], Parra, V.[1], Rodríguez, A.E.[1], Quiroga, C.[1], Paredes, F.[1], Quest, A.F.[1,2], Lavandero, S.[1,2]. FONDAP CEMC, Facultad de Ciencias Químicas y Farmacéuticas/ Facultad de Medicina, Universidad de Chile[1], ICBM, Facultad de Medicina, Universidad de Chile[2].

The endoplasmic reticulum (ER) is a crucial organelle for protein, lipid and calcium homeostasis. In response to perturbations, the ER acts as a stress sensor by triggering a variety of signal transduction pathways. As part of this response, ER contacts with mitochondria increase to facilitate calcium transfer between both organelles and stimulate mitochondrial bioenergetics. The protein kinase mTOR is a key regulator of cellular metabolism, but whether it participates in the control of organelle exchange is not known. Here we addressed this question in HeLa cells, a model previously established in our laboratory to evaluate alterations in mitochondria-ER interactions. Both rapamycin-mediated mTOR inhibition and tunicamycin-induced ER stress increased inter-organelle contacts, as assessed by fluorescence confocal microscopy. These changes were paralleled by a higher rate of oxygen consumption, suggesting an increase in mitochondrial bioenergetics. Interestingly, both treatments induced distinct spatial distribution and morphological patterns of mitochondria. While rapamycin led to a more interconnected and evenly distributed mitochondrial network, tunicamycin treatment resulted in redistribution of mitochondria to the perinuclear region and local perturbations in network continuity. These observations suggest that both treatments affect a common global mechanism, but also engage distinct local signaling pathways. Future experiments will seek to determine the consequences of these differences for mitochondrial physiology and the signaling pathways involved in their regulation. RB, AR, CQ, FP, VP are funded by CONICYT. FONDAP 15010006; FONDECYT 1080436.

A novel mechanism for mTOR-dependent mitochondrial dynamics regulation. Verdejo, H.[1,2], Parra, V.[3], Kuzmicic, J.[3], Lavandero, S.[3]. Division de Enfermedades Cardiovasculares Facultad de Medicina Pontificia Universidad Catolica de Chile[1], Programa Doctorado Ciencias Medicas Facultad de Medicina Pontificia Universidad Catolica de Chile[2], Centro FONDAP Estudios Moleculares de la Célula Facultad de Ciencias Químicas y Farmacéuticas/Facultad de Medicina Universidad de Chile[3].

The metabolic sensor mTOR has a key role in the integration of several signaling pathways involved on cell metabolism. Our previous work indicates that insulin, a physiological stimulus involved in mTOR signaling, promotes mitochondrial fusion and respiration; however, the role of mTOR on mitochondrial dynamics is still poorly understood. The aim of the present work was to evaluate the implication of mTOR on the control of mitochondrial dynamics and metabolism in cultured rat cardiomyocytes. The results show that mTOR inhibitor rapamycin triggers mitochondrial fission within 6 h as evidenced by a marked increase in the number of mitochondria and a decrease in average mitochondrial volume, assessed by confocal microscopy in mitotracker green-stained cells. Such increase did not depend on mitochondrial biogenesis, assessed by SDS-PAGE against the constitutive mitochondrial protein Hsp70. The specific and irreversible inhibitor for mTORc1 complex Torin-1 replicated the mentioned effects on mitochondrial morphology and function. Additionally, mitochondrial fission induced by rapamycin was associated with marked reductions in oxygen consumption, ROS content and ATP levels. These changes were also linked to increased degradation of the inner mitochondrial membrane GTPase Opa1, involved in mitochondrial fusion. Interestingly, the inhibition of the zinc-dependent metalloproteases by o-phenantrolin abrogated mTOR-induced Opa1 processing, reverting both the morphological and functional effects induced by rapamycin. Taken together, these data suggest a new regulatory mechanism for mitochondrial dynamics and metabolism depending on mTOR-dependent Opa1 processing. FONDAP 15010006. HEV, VP and JK hold PhD fellowship from CONICYT.

The PIP5K1 and 2 enzymes are required for the normal reproductive development in *Arabidopsis thaliana*. Ugalde, J.M.[1], Tejos, R.[2], Friml, J.[2], León, G.[1]. Laboratorio de Reproducción y Desarrollo de Plantas, Centro de Biotecnología Vegetal, Universidad Andrés Bello.[1], Department of Plant System Biology, VIB Research Institute and Gent Universitiet, Belgium.[2].

The Phosphatidyl Inositols (PtdIns) are membrane lipids located on the cytoplasmic side of animal and vegetal cells, they have an important role in the integration of several transduction signaling pathways. The enzymes PIP5K1 and 2 belong to the family of enzymes that phosphorilate the carbon 5 of the inositol ring, using preferentially PI(3)P or PI(4)P as substrate to produce PI(3,5)P2 or PI(4,5)P2. These proteins have an 86% of identity on their sequences and had been identified as important in the transduction signaling pathway generated by the Auxin hormone in *Arabidopsis thaliana*. Single mutant plants for these genes show mild defects associated to reproductive development. In contrast, we?ve found that double mutant plants show several problems associated to the reproductive development, including production of dead pollen grains, defects in the ovules, reduction in the seed set and alterations in the early embryo development. Furthermore, we detected alterations in the pollen tube elongation, suggesting an important role of these enzymes for the correct polar growth of the pollen tube. We?ll analyze the dynamic of auxin in pollen and pollen tubes of wild type and mutant plants using transgenic lines expressing the GUS reporter gene controlled by the auxin responsive promoter DR5. Altogether, our results suggest an important role of Auxins in the reproductive development trough PtdIns in Arabidopsis. Funded by Fondecyt 11080037 and Odysseus program, FWO.

LLP, a plasma membrane lectin induced by salicylic acid and involved in the defense response against *Pseudomonas syringae* in *Arabidopsis thaliana*. Armijo, G.[1], Seguel, A.[1], García, C.[1], Salinas, P.[1], Leiva , D.[1], Holuigue, L.[1]. Departamento de Genética Molecular y Microbiología, Facultad Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago de Chile. [1].

Plants have complex systems to respond and adapt to stressful conditions. These mechanisms are mainly mediated by phytohormones, such as salicylic acid (SA). The role of SA has been principally characterized in the defense response against biotrophic pathogens. Previously, we identified a group of genes activated by SA in *Arabidopsis thaliana*, among which LLP (lectin-like-protein) had the highest activation level. LLP protein shows similarity to the legume lectin family and so far has not been associated to any biological function. To determine the LLP function in plant defense against biotrophic pathogens, we used *Pseudomonas syringae* as one of the best models for these interactions in Arabidopsis. First, we inoculated wild type and sid2 plants (mutants in SA biosynthesis) with *Pseudomonas* strains that trigger distinct plant responses. We found that LLP is activated by avirulent strains by a SA-dependent mechanism. Then, we developed transgenic lines over-expressing LLP fused to GFP or c-Myc and characterized mutant lines null for LLP. We analyzed 35S::LLP-GFP lines by confocal microscopy and detected the fusion protein located in the plasma membrane. Later, we evaluated the proliferation of *Pseudomonas* in over-expressor and mutant lines. We found that over-expression of LLP reduced *P. syringae*-Avr-Rpm1 proliferation and increased cell death in inoculated tissues. These results strongly suggest that LLP is involved in the defense response to this bacteria. Currently we are looking for specific interactors to gain further insights in LLP specific function. FONDECYT (1100656) and Millennium-Nucleus PFG (P10-062-F).

GENE EXPRESSION I

Identification and functional characterization of *ERF115* gene coding for a transcription factor involved in tolerance to high salinity stress in *Arabidopsis thaliana*. **León, L.**[1], Casaubon, M.J.[1], Villaroel, E.[1], Holuigue, L.[1]. Plant Molecular Biology Laboratory and Millennium Nucleus for Plant Functional Genomics, Department of Molecular Genetics and Microbiology, Pontifical Catholic University of Chile[1].

Plants are constantly exposed to different conditions of biotic and abiotic stress. There is a partial overlap in the physiological and genetic responses of plants to these different stressful conditions, which allows identifying genes that are activated by different or specific stress conditions. Among abiotic stress conditions, high salinity becomes very important because of its wide distribution and the negative effects on plant growth and production. With the purpose to identify transcription factors that are induced specifically in roots under salt treatments, an *in silico* analysis of the Arabidopsis transcriptome was first performed using public available microarray data. Differentially expressed genes under high salinity stress were identified and clustered according to their expression profiles. We performed a gene network analysis of genes induced in roots by salinity and we selected the *ERF115* transcription factor that showed the greatest number of interactions in the network. Using RT-qPCR we proved that *ERF115* is selectively induced in roots by high salt treatments. Moreover, we identified and characterized a homozygous insertional mutant line *erf115/erf115*, which is null for *ERF115* expression. Phenotypic analyses showed that the mutant line had a dwarf phenotype compared to WT plants under salinity stress. Using network analyses we found 69 putative targets of *ERF115*, which have overrepresented the gene ontology term "*transport compounds, protein transport and transport ATPases*". These results suggest an important role for *ERF115* in the tolerance to salinity stress by inducing genes involved in transport. FONDECYT-1100656, MN-PFG-(P10-062-F).

Optimizing homologous recombination in slow growing mycobacteria. **Gonzalez, P.**[1,2,3,4,5], Jain, P.[4,5], Hsu, T.[4,5], Jacobs Jr., W.[4,5]. Millennium Institute on Immunology and Immunotherapy. Departamento de Genética[1], Molecular y Microbiología, Facultad de Ciencias Biológicas. Pontificia Universidad Católica de[2], Chile, Santiago, Chile.[3], Howard Hughes Medical Institute and Department of Microbiology and[4], Immunology, Albert Einstein College of Medicine, Bronx, NY, USA[5]. Sponsored by A. Kalergis.

Mycobacterium tuberculosis (Mtb), the etiologic agent responsible for tuberculosis is the leading cause of death by communicable diseases worldwide, killing over 2 million people eachyear. Furthermore, nearly one third of the world population is infected with this bacterium. The current vaccine to combat Mtb, BCG (Bacille Calmette-Guerin) has proven to be ineffectiveagainst pulmonary tuberculosis. Although somewhat toxic to the host, drug treatment has proven, in most cases to be effective (84%). However, multi-drug and extensively drug-resistantresistant Mtb strains, which are virtually untreatable with current chemotherapies are emerging at important growth rates worldwide. Thus, development and identification of novel vaccine and drug alternatives are urgently needed. Importantly, analysis of Mtb strains harboring gene deletions can promote these processes. However, traditional strategies used to generate these strains introduce polar effects masking the physiologic function of genes. Currently we are engaged on an approach to generate a 4,400 antibiotic-resistance less, single gene deletion Mtb strain library. Because homologous recombination in mycobacteria is intrinsically inefficient, we have developed novel strategies to optimize this process. Some of these strategies include: 1) use of mycobacteriophages to efficiently deliver allelic exchange substrates into the bacterium, 2) use of E. coli prophage Rac, RecE and RecT homologues in delta-recD mycobacteria strains, 3) a method for linearizing allelic exchange substrates within the bacterium and 4) optimization of codon usage for efficient counterselection during antibiotic unmarking.

Annotation of putative AraC/XylS-family transcription factors with unknown function. **Schüller, A.**[1,2], 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[2].

AraC/XylS is a family of transcriptional regulators found in bacteria controlling the expression of genes with diverse biological functions involved in metabolism, stress response and virulence. To identify members of this family we performed structural alignments of proteins contained in the Protein Databank with the three-dimensional structure of MarA - an AraC-type transcriptional activator. Four putative AraC-family structures with high structural similarity and unknown biological function were identified. Next, we generated a multiple sequence alignment of non-redundant AraC-family sequences from the UniProt database and constructed a phylogenetic tree using the Maximum Likelihood optimization criteria. Seven well-defined groups of sequences could be identified in this family. The four structures with unknown function are related to members that were previously identified as regulators of metabolism. A sub-family of factors regulating stress response showed a high degree of conservation. Structural analysis identified a tryptophan residue engaged in a specific interaction with a cytosine nucleobase of the DNA binding sequence. Tryptophan has a low statistical propensity to interact with DNA and both residues are highly conserved. Thus, this interaction likely contributes to binding specificity of the stress response sub-family. Absence of this interaction from any of the four putative AraC-family structures indicates a biological role other than stress response. Acknowledgements: A.S. is grateful for a FONDECYT postdoctoral research grant (3110009) and A.W.S. is grateful for a CONICYT postgraduate scholarship. This research was funded by grants from FONDECYT (1110400) and ICM (P09-016-F).

Full-atom structure-based prediction of transcription factor binding sites. **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 paramount 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.Two main approaches to predict protein binding sites in the DNA have been reported: 1) Sequence-based methods, that use patterns or profiles coming from sequence alignments (e.g. consensus sequences, WebLogos) and 2) Structure-based methods, that use structural information coming from protein-DNA complexes solved by X-ray crystallography or NMR. The sequence-based methods are the most popular because of the simplicity in their use and implementation. However, these methods are not very accurate, exhibiting poor sensitivity/specificity trade offs.In this work, we present the development of novel statistical potentials that describe the protein-DNA interactions, which are used to estimate the stability of proteins-DNA complexes from the atomic coordinates of their 3D structures. Here we show that the combined use of these potentials and software for the 3D modelling of protein-DNA complexes allowed us to recover in a large degree the known experimental binding sites for several transcription factors.ACKNOWLEDGEMENTSThis research was funded by grants from FONDECYT (1110400) and ICM (P09-016-F).

The unfolded protein response has a circadian component in *Neurospora crassa*. **Goity, A.**[1], Montenegro-Montero, A.[1], Larrondo, L.F.[1]. Depto. Genética Molecular y Microbiología, Facultad Ciencias Biológicas, Pontificia Universidad Católica de Chile.[1].

The unfolded protein response (UPR) is a stress pathway triggered by an excess of misfolded proteins in the endoplasmic reticulum. The mechanisms involved in this stress circuit are widely conserved among eukaryotes. Due to the high protein secretion capacity displayed by filamentous fungi, the study of secretion pathways and its regulation in these organisms is of special interest. The ascomycete *Neurospora crassa*, a model filamentous fungus, has been instrumental for the study of a variety of complex molecular processes, including circadian rhythms. Particularly, *Neurospora* has advanced our understanding of the molecular basis of these daily oscillations in a wide range of organisms, including mammals. The circadian clock imposes rhythmic regulation upon different processes in the cell, so that organisms can anticipate daily changes in environmental conditions. Our lab is interested in transcriptional networks emerging from the circadian clock in *Neurospora,* and in this work, we characterized the UPR system in this organism and examined the mechanisms involved in the transcriptional regulation of its key components. We show that the basic aspects of the UPR are conserved with other organisms, but notably, that the circadian clock appears to controls the expression of several of its components. We are currently trying to decipher the relevance and implications of this circadian control of UPR on the way *Neurospora* copes with environmental stress. FONDECYT 1090513, CONICYT.

Transcriptional regulation of an embryogenesis-specific iron-sulfur protein isoform of *Arabidopsis thaliana* mitochondrial complex II. **Restovic, F.**[1], Roschzttardtz, H.[1], Vásquez, M.[1], Gómez, M.I.[1], Vicente-Carbajosa, J.[2], Jordana, X.[1]. Pontificia Universidad Católica de Chile[1], Universidad Politécnica de Madrid[2].

Complex II (SDH) is part of both the citric acid cycle and the respiratory electron transfer chain. Three nuclear genes encode its iron-sulfur subunit (SDH2). One of them, SDH2-3, is specifically expressed in the embryo during seed maturation, suggesting that it may have a role during embryo maturation and/or germination. Due to the importance of embryogenesis and germination, we have undertaken the study of the function and expression of this gene. We have determined that mutants present lower SDH activity in mature seeds, a delay in germination and shorter hypocotyls in the dark. We have also identified promoter cis elements (ABRE and RY) that are necessary but not sufficient for high embryo expression, established through the study of mutants the importance of B3-type transcription factors (ABI3, FUS3 and LEC2), and suggested the participation of bZIP transcription factors by in vitro DNA binding assays. We have now deepened the characterization of the SDH2-3 transcriptional regulation, delimiting the promoter region necessary and sufficient for high seed expression and uncovering putative additional cis elements. Furthermore, we have confirmed that bZIP transcription factors are involved in its regulation, and showed by transient expression analysis in Arabidopsis protoplasts that bZIP (bZIP10, bZIP25 and bZIP53) and B3-type (ABI3 and FUS3) synergistically activate SDH2-3 transcription. It is noteworthy that a gene encoding a subunit of a metabolic enzyme is controlled in such a tight way, with an expression profile resembling that of seed storage proteins. Fondecyt1100601. Milenio-P10062F.

Transcriptional regulation of trafficking-genes in *Arabidopsis thaliana*. **Pizarro, L.**[1,2], Vergara, A.[1], Gutierrez, R.[3], Rojas-Pierce, M.[4], Norambuena, L.[1,2]. Plant Molecular Biology Laboratory. Faculty of Science. University of Chile.[1], Plant Cell Biotechnology Millennium Nucleus.[2], Plant System Biology Lab. Molecular Genetic and Microbiology Department. Faculty of Biological Science. Pontifical Catholic University of Chile.[3], Department of Plant Biology. North Carolina State University. Raleigh, NC.[4].

The localization of proteins in their destination compartments is essential for physiological protein function therefore sorting accuracy is fundamental for cell functioning. In eukaryotic cells, the endomembrane system (ES) has a central role ensuring correct protein destination of enormous amounts of proteins. The regulation of ES protein trafficking has been described mostly at post-translational level. However, there is raising evidence that trafficking is regulated at transcriptional level by means of changes in gene expression of trafficking-genes. To search for putative transcription factors (TF) involved in trafficking regulation in Arabidopsis thaliana we have constructed the Trafficking Regulatory Network. Its building was considering binding-site enrichment in the promoter of trafficking-genes and co-expression degree between TF and trafficking-genes. We had ranked and selected seven TF as putative trafficking transcriptional regulators, called TFES. In order to analyze the transcriptional response of trafficking-genes, the response of trafficking-genes in seedlings under trafficking-disruption drug treatments was determined by qRT-PCR. We found four trafficking-disruption responsive genes, Sar1 and cargo binding proteins. To test the TFES as trafficking transcriptional regulators we have chosen in vivo loss-function analysis. Interestingly a mutant of TFES1 has abnormal levels of sar1 transcript, suggesting that TFES1 regulates sar1 expression. In addition confocal microscopy analysis showed that tfes1 mutant had an abnormal ER shape. Those results suggest that TFES1 could be involved in ER architecture through the transcriptional regulation of trafficking genes such as Sar1. FONDECYT11080240, ICMP06-065-F, Universidad de Chile Short-Term Scholarship and Conicyt-Chile Fellowship.

The transcription factor CREB binds to the proximal region of the human RIC-8B promoter. **Maureira Moya, A.**[1], María Victoria, H.[1], José Leonardo, G.[2], Juan, O.[1]. Laboratorio de Genética Molecular, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción. [1], Laboratorio de Regulación Transcripcional, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción[2].

Ric-8B is a highly conserved guanine nucleotide exchange factor (GEF) for $G_{\alpha}s$, which presents a restricted expression profile in brain, probably driven by response elements present in its promoter region. The cAMP Response Element Binding Protein (CREB) is a transcription factor associated to the expression of several neuronal genes involved in cell differentiation and learning-memory processes. Therefore, we performed a bioinformatic analysis over the proximal region of the human Ric-8B (hRic-8B) promoter using different programs, which showed the presence of two CRE motifs. Due to the absence of a TATA-box in this promoter, these CRE elements should not respond to increased levels of cAMP. However, it has been reported that in some genes, CRE sites can maintain a basal promoter activity in a TATA-less context. In order to evaluate in vivo the role of CRE elements of the hRic-8B promoter, we performed a Chromatin Immuno-Precipitation (ChIP) assay. Using human HEK-293T and neuroblastoma SH-SY5Y cells, we demonstrated the binding of CREB to the hRic-8B promoter CRE elements. Furthermore, we confirmed in vitro, through Electrophoresis Mobility Shift Assay (EMSA) using the hRic-8B promoter CRE sites as radiolabeled probes and nuclear extracts from HEK-293T and SH-SY5Y cell lines, the functionality of these CRE elements. All these data suggest a role of cAMP signaling in hRic-8B expression.FONDECYT GRANT 1090150.

GENE EXPRESSION II

Systems approaches map regulatory networks downstream of the auxin receptor *AFB3* in the nitrate response of *Arabidopsis thaliana*. **Vidal, E.A.**[1], Moyano, T.C.[1], Riveras, E.[1], Gutiérrez, R.A.[1]. FONDAP Center for Genome Regulation, Millennium Nucleus Center for Plant Functional Genomics, Departamento de Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile[1].
Nitrogen (N) is an essential macronutrient available to plants mainly as nitrate in agricultural soils. Genomics analyses have now provided thousands of nitrate-responsive genes in Arabidopsis. Recently, systems approaches have been utilized to identify discrete molecular networks that plants utilize to adapt metabolic, cellular and developmental processes to changing N availability. The challenge now is to understand the molecular mechanisms underlying N-regulation of gene networks and bridge the gap between N-sensing, signaling and downstream physiological and developmental changes. Using next generation sequencing technologies and bioinformatics analysis of the sequence data, we identified a nitrate-responsive microRNA/*TARGET* module consisting of miR393 and the *AFB3* auxin receptor. This module represents a coordinated regulatory feedback loop that is induced by nitrate and repressed by N forms produced by nitrate reduction and assimilation, regulating root system architecture (RSA) in response to internal and external nitrate availability. We used an integrated genomics, bioinformatics and molecular genetics approach to identify regulatory networks acting downstream miR393/*AFB3* that control RSA in response to nitrate. Our results point at a specific role for *AFB3* in the root nitrate response and identified a highly connected regulatory network that controls RSA downstream of this auxin receptor. Acknowledgements: FONDAP Center for Genome Regulation, Millennium Nucleus Center for Plant Functional Genomics, Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT) 1100698, Proyecto de Inserción PSD74 CONICYT.

Identification of two proteins involved in the control of rhythmic gene expression in *Neurospora crassa*. **Olivares-Yañez, C.**[1], Muñoz, F.[1], Larrondo, L.F.[1]. Depto. Genética Molecular y Microbiología, Facultad Ciencias Biológicas. Pontificia Universidad Católica de Chile[1].
The filamentous fungus *N. crassa* is a model organism for the study of circadian rhythms. Circadian clocks are generally described as composed of three elements: input pathways, a central oscillator and output pathways. Even though the mechanisms underlying the central oscillator and how it senses environmental cues has been extensively studied, little is known about how the central clock coordinates the expression of several rhythmic processes (output). By using a reverse genetics approach and a luciferase-based high-throughput screening, we have identified two genes involved in the regulation of rhythmic gene expression downstream from the central oscillator. Both genes, *ptc-1* and *sub-1*, are clock-controlled themselves and the circadian expression of the former is regulated by SUB-1. By using gene expression microarrays, we have observed that in the absence of either of these two regulators, the expression of approximately 100 genes is compromised. Transcriptional fusions of the promoter region of a subset of these genes and a fully codon optimizated luciferase reporter gene have shown that they are also *ccgs*. Here we describe that SUB-1, a GATA transcription factor previously described as a light regulated and regulator gene, also participates in the regulation of circadian output. PTC-1, a conserved developmental regulator in filamentous fungi that has not being associated to circadian output, is also described herein as to be involved in the regulation of circadian gene expression. Fondecyt 1090513, CONICYT.

Participation of light and the circadian clock in the pathogenicity of *Botrytis cinerea*. **Hevia, M.**[1], Canessa, P.[1], Larrondo, L.F.[1]. Depto. Genética Molecular y Microbiología, Facultad Ciencias Biológicas, Pontificia Universidad Católica de Chile.[1].
Botrytis cinerea is a necrotrophic fungus that infects over 200 plant species. In Chile, it produces significant damage in agribusiness infecting pre and post harvest fruits. Interestingly, it has been documented that the interaction between a pathogen and its host is a process modulated by light and possibly, the circadian clock. Nevertheless, nothing is known about the molecular mechanisms behind the aforementioned interaction in necrotrophic fungi. In order to determine this phenomenon, we have characterized the circadian clock and the transcriptional response mediated by the blue light photoreceptor White Collar 1 (WC-1) in *B. cinerea* . In *Neurospora crassa*, the circadian clock is composed by the FRQ protein and a transcriptional complex formed by WC-1 and WC-2 (WCC). *In silico* analysis have shown that these genes are present in the Botrytis genome. Using a translational luciferase reporter, we have observed oscillatory levels of the FRQ protein, while RT-qPCR experiments have indicated that there is also an acute transcriptional response to light mainly dependent on the clock components. This response is dramatically inhibited in a WC-1 delta strain and the growth and sporulation banding in a light-dark cycle is no longer observed. Pathogenicity assays are been conducted in order to determine the impact of the mentioned delta strain. Fondecyt 1090513, ICGEB-CRP CHI09-02, IFS AC/20198, CONICYT.

Herp stimulates cytoprotection against oxidative stress by regulating intracellular calcium. **Paredes, F.**[1], Quiroga, C.[1], Jaimovich, E.[1,2], Lavandero, S.[1,2]. FONDAP CEMC, Facultad de Ciencias Química y Farmacéuticas/Facultad de Medicina, Universidad de Chile[1], ICBM, Facultad de Medicina, Universidad de Chile[2].
The endoplasmic reticulum (ER) protein Herp is induced in response to ER stress, osmotic stress or deregulation of Ca^{2+} homeostasis. Although Herp function is not completely understood, this ER protein has been associated with ER associated protein degradation (ERAD), regulation of intracellular calcium, cytoprotection, mitochondrial dysfunction and autophagy. Our aim was to investigate whether oxidative stress induces Herp as a cytoprotective compensatory mechanism and the role of Ca^{2+} in this response. Our results depicted that Herp levels increased near 5-fold in Hela cells treated with H_2O_2 (500 uM) for 0-1 h. The maximal Herp expression was attained after 30 min with H_2O_2. Herp knock down cells exhibited more cell death compared to controls assessed both PI incorporation/flow cytometry and trypan blue staining. These evidences showed that Herp has protective effect against oxidative stress. The intracellular Ca^{2+} chelator BAPTA-AM prevented the increase in cell survival observed in Herp knock down cells treated with H_2O_2, suggesting a role of calcium in the process. In conclusion, our data show that Herp protects HeLa cells from oxidative stress by regulating intracellular calcium levels.FONDAP 1501006 (SL, EJ). Becario Doctorado CONICYT (FP).

Expression of a Mg^{2+}-dependent HIV-1 RNase H in a single polypeptide for drug screening. Vargas, D.A.[1], Castillo, A.E.[1], Roth, M.J.[2], Leon, O.[1]. Programa de Virología, ICBM, Facultad de Medicina. Universidad de Chile.[1], UMDNJ-RWJMS, Biochemistry, Piscataway, NJ.[2].

The HIV reverse transcriptase (RT) has been a major target for antiviral drug development. This enzyme is a heterodimer consisting of p66 and p51 subunits and contains two enzymatic activities: DNA polymerase (RNA and DNA-dependent) and RNase H, both essential for viral replication. All clinically approved RT inhibitors target the polymerase site, because is difficult identify inhibitors that binds specifically to the RNase H site, since both sites are interdependent. In this report, a single polypeptide of HIV-1 RT without the DNA polymerization site was made by joining of p51 subunit, by a linker, to the thumb (T), connection (C), and RNase H (R) domains of p66. This p51-G-TCR construct was purified from the soluble fraction of a RNase H deficient *E.coli* strain MIC2067(DE3). The purified protein p51-G-TCR behaves as a monomer in solution as judged by dynamic light scattering and displayed Mg^{2+}-dependent activity using a fluorescent nonspecific assay. Moreover, p51-G-TCR shows the same cleavage pattern as HIV-1 RT on specific substrates that mimic the tRNA primer removal. The RNase H of the p51-G-TCR construct and HIV-1 RT had a similar Km for an RNA/DNA hybrid substrate and showed similar inhibition kinetics with known inhibitors of the HIV-1 RT RNase H. The ability of this single polypeptide to support Mg^{2+}-dependent RNase H activity makes it an attractive reagent for antiviral drug screening targeting the RNase H domain. FONDECYT 1080137.

The Andes Hantavirus NSs protein is expressed from the viral small mRNA by a leaky scanning mechanism. Solis, L.[1], Vera, J.[1], Pino, K.[1], López-Lastra, M.[1]. Laboratorio de Virologia Molecular, Centro de investigaciones Medicas, Facultad de Medicina, Pontificia Universidad Catolica[1].

The Andes Virus (ANDV) small messenger RNA (SmRNA), that encodes the nucleocapsid (N) protein, exhibits an overlapping (+1) open reading frame (ORF), predicted to encode a putative nonstructural protein (NSs). In this study we evaluated if the ANDV NSs protein was indeed expressed in the context of a viral infection. Our results show that the NSs protein is expressed in ANDV infected cells. Interestingly, the NSs protein localizes to the cytoplasm and exhibits a granular staining pattern. Next we conducted experiments to understand the mechanism of translation initiation driving NSs protein expression from the SmRNA. Results suggest that recognition of the NSs initiation codon is mediated by ribosomal subunits that have bypassed the upstream the N protein initiation codon through a leaky scanning mechanism. LS holds a CONICYT doctoral fellowship. This study was funded by the Instituto Milenio Inmunología e Inmunoterapia (IMII) and a NIH/ICIDR (2U01AI045452-11) grant.

Regulation of non-coding mitochondrial RNA by a High Risk Human Papillomavirus (HPV) protein. Campos, A.[1,2,3], Varas, M.[1,2,3], Burzio E., L.[1,2,3], Villota, C.[1,2,3]. Andes Biotechnologies S.A[1], Fundación Ciencia para la Vida[2], Facultad de Ciencias, UNAB[3]. Sponsored by J. Villegas.

Our group has described a novel family of human non-coding mitochondrial RNAs (ncmtRNAs), which display a differential expression pattern according to proliferative status. Normal proliferative cells express two transcripts known as Sense-ncmtRNA (SncmtRNA) and Antisense-ncmtRNA (ASncmtRNA). Tumor cells, on the other hand, express SncmtRNA but down-regulate ASncmtRNA. In order to explore the mechanisms involved in ASncmtRNA down-regulation during immortalization and transformation, we have used human keratinocytes immortalized with Human Papillomavirus (HPV) 16 and 18 as a model, where we found that the expression pattern of the ncmtRNAs is the same as tumor cells. We previously established that the HPV oncoproteins E6 and/or E7 are not involved in the establishment of this phenotype. Here, we evaluate the involvement of HPV oncoproteins E5, E4 and E2 in down-regulation of ASncmtRNA. We found that E5 knockdown with shRNA in immortalized cells did not induce changes in the expression of ASncmtRNA. On the other hand, E4 knockdown induced expression of ASncmtRNA but, contrary to what we expected, exogenous expression of E4 in normal keratinocytes had no effect on expression of ncmtRNAs. These results led us to evaluate the E2 protein, which shares nucleotide sequence with E4 and its gene expression is also silenced by shRNAE4. In agreement to our hypothesis, when we knocked-down E2 in immortalized cells, expression of ASncmtRNA was reestablished. This suggests a role for HPV E2 in down-regulation of ASncmtRNA at a very early stage in cell immortalization. FONDECYT 11090060, DI06-09/R.

Genetic association between host IL28B genotype and extrahepatic HCV infection. Angulo, J.[1,2], Pino, K.[1], Biel, F.[3], Soza, A.[3], López-Lastra, M.[1]. Laboratorio de Virología Molecular, Instituto Milenio de Inmunología e Inmunoterapia (IMII), Centro de Investigaciones Médicas, Facultad de Medicina, Pontificia Universidad Católica de Chile[1], Programa de Doctorado en Microbiología (USACH)[2], Departamento de Gastroenterología, Facultad de Medicina, Pontificia Universidad Católica de Chile[3].

Infection of peripheral blood mononuclear cells (PBMC) by the hepatitis C virus (HCV) might represent a viral strategy to evade the host immune response. Extrahepatic replication might also allow HCV to escape antiviral therapy. To evaluate the impact of PBMC infection on the outcome of antiviral therapy, we looked at the presence of HCV-RNA in PBMC of chronically infected patients undergoing peginterferon/ribavirin treatment for 48 weeks. Our results showed a negative correlation between the presence of HCV-RNA in PBMC (at week 0) and the outcome of antiviral therapy (measured as viral load 24 weeks after finishing treatment). Thus, patients with PBMC positive to HCV-RNA were less likely of achieving a sustained virological response. However, not all chronically HCV infected patients exhibited viral-RNA in PBMC, suggesting that host factors might be involved in extrahepatic infection. Recent genome-wide association studies report that the G allele of single nucleotide polymorphism (SNP) rs8099917 is associated with antiviral therapy failure. To determine the effect of the rs8099917 variation in PBMC infection, we genotyped this SNP in a cohort of 83 infected patients. Our results revealed an association between the G allele (GT/GG genotype) and the occurrence of HCV-RNA in PBMC. Taken together, these results suggest that PBMC infection by HCV is a negative predictor of treatment response. In addition, the presence of G allele of rs8099917 seems to determine the susceptibility of PBMC to HCV infection.CONICYT Scholarship ProyectoPuente/DIPUC-Escuela de Medicina, PUC, Alejandro Soza, 2011.

POSTER SESSIONS EXHIBITION

1.RNA aptamers: a new approach for the diagnosis and therapy of envenomation by *Loxosceles* spiders. **Constenla-Muñoz, C.**[1], **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].

Loxoscelism is the clinical condition arising from the bite of *Loxosceles* spiders. It is characterized by necrotizing skin lesions (dermonecrotic loxoscelism) but may also include severe systemic reactions (viscerocutaneous loxoscelism) which can even lead to death. *Loxosceles laeta*, endemic to Chile, is the most dangerous *Loxosceles* species. Its venom is a complex mixture containing various isoforms of sphingomyelinase D (SMD) which can elicit biological effects similar to those of whole venom. Treatment for loxoscelism is mainly palliative and there are no diagnostic means available. Aptamers are generally small oligonucleotides capable of binding specifically to a protein. Thus, RNA aptamers which bind to SMDs have potential use both as molecular agents to diagnose the spider bite and, if capable of enzymatic inactivation, as drugs to treat loxoscelism. Aptamers may be obtained by iterative *in vitro* selection from a pool of $>10^{14}$ random RNA molecules. Two *Loxosceles laeta* SMDs (LI1 and LI2) were synthesized by cDNA assembly and cloned in a vector providing a 6-His tag. Proteins were produced in *Escherichia coli* and purified by Ni-NTA chromatography. A combinatorial pool of RNAs (101 nt) was mixed with LI1 or LI2 and filtered through a nitrocellulose membrane to select RNAs bound to the proteins. RNA was recovered and subjected to ~10 successive rounds of reverse transcription, PCR, *in vitro* transcription and selection to obtain pools enriched in competent RNAs. Binding affinity and inactivation capacity of selected pools will be presented. (FONDECYT 1100209).

2.Dopamine and spermatozoa; a relationship to be understood. **Urra, J.**[1], Villarroel, F.[2], Lopez, C.[2], Rodríguez-Gil, J.E.[3], Ramírez, A.[4], Concha G, I.I.[2]. Instituto de Bioquímica y Microbiología, Instituto de Ciencia Animal, Universidad Austral de Chile[1], Instituto de Bioquímica y Microbiología, Universidad Austral de Chile[2], Unitat Reproducció Animal, Universitat Autonoma de Barcelona, España[3], Instituto de Ciencia Animal, Universidad Austral de Chile[4].

Dopamine (DA) is of great scientific interest, either because of the physiological activities in which it participates, diseases in which it is involved or drug dependence. DA has been reported in male and female reproductive tract. However its role remains unknown. In our laboratory, we reported the functional expression of D2 receptors in spermatozoa, suggesting that DA acts as a physiological modulator of viability, capacitation, and sperm motility. Low-concentration DA (100 nM and 10 uM) increased total and progressive motility of sperm, but high concentrations of DA (1 mM) decreased both tyrosine phosphorylation and motility in capacitation assays. In this study we showed the expression and function of proteins of the catecholaminergic system (tyrosine hidroxilase, L-DOPA decarboxilase, monoamine oxidase), and monoamine transporters (dopamine, noradrenalin and serotonin). The results demonstrated, by immunofluorescence and Western blot analysis, the presence of components of the catecholaminergic system in human, bovine and equine spermatozoa. DA (1 mM) did not affect viability but inhibited total and progressive motility, situation that was reversed when using inhibitors of DA transporter. These results may explain reproductive disorders linked to syndromes such as cocaine or amphetamine addiction as well as for antipsychotic drug use. (FONDEF D08I1076, FONDECYT 1110508, DID-UACh D2010-01, Beca CONICYT JU, Escuela Graduados Facultad de Ciencias Veterinarias, Dirección de Postgrado UACh, DID-UACh).

3.Down expression of insulin receptor in human renal cortex of diabetic patients. **Gatica, R.**[1,2,3,4], Kairath, P.[2], Caelles, C.[4], Slebe, J.C.[2], Yañez, A.[2]. Escuela de Graduados Facultad de Ciencias Veterinarias [1], Instituto de Bioquímica y Microbiología Universidad Austral de Chile [2], Universidad San Sebastián Sede Puerto Montt[3], Institute for Research in Biomedicine Barcelona España. [4].

Insulin is an essential hormone for energy management and its binding to the insulin receptor leads to a cascade of intracellular signalling events, which lead to a regulation of multiple biological processes such glucose metabolism. The insulin receptor (InsR) is a member of the receptor tyrosine kinase superfamily, and an integral glycoprotein of the plasma membrane in most mammalian cells. Here, we studied the expression, protein levels and localization of InsR in human renal cortex of type 2 diabetic patients. Western blot analysis showed a significant reduction of the InsR subunit beta level and phosphorylation of GSK3beta in diabetic human renal cortex and down regulation of proreceptor in Diabetic human renal proximal tubule (DHRPTC). Furthermore, InsR immunoreactivity was localized in basolateral and apical plasma membrane of renal cortex tubule and loop of Henle in normal patients and this pattern changed to low immunoreactivity in type 2 diabetic patients. This differential subcellular distribution and expression suggests the involvement of the InsR in the metabolic changes of the DHRPTC of diabetic patients and its implication in the diabetic nephropathy (Foundation M. Botin; DID-UACh: 2006-19; FONDECYT 1090694).

4.Transcriptomic network analysis of *Arabidopsis thaliana* reveals specific gene regulation under cold, salt and UV-B stress conditions. **Sagredo C.E.**[1,2], Espinoza, J.A.[2,3], Bizama, C.[2], Cabrera, G.[2], Gutiérrez Moraga, A.[2,3]. Carrera de Biotecnología, Universidad de La Frontera,Temuco, Chile[1], VentureL@b, Universidad Adolfo Ibáñez, Chile.[2], Programa de Doctorado en Ciencias Mención Biología Celular y Molecular Aplicada, Universidad de La Frontera,Temuco, Chile[3].

In this work, a transcriptional network analysis was performed in order to identify gene co-expression groups (clusters) which are specifically expressed in ultraviolet light B (UV-B), salt or cold stress in *Arabidopsis thaliana* plants. For this purpose, public datasets (GEO5626, GEO5623 and GEO5621, Affymetrix(r) Arabidopsis ATH1 platform) containing expression values of *A. thaliana* leaves under UV-B, salt and cold stress during 24 hours were used. This analysis was carried out using Biolayout Express^{3D} software and the gene ontologies enrichment was performed using DAVID v6.7. We found twenty seven clusters which their expression profiles changed only under UV-B, salt or cold stress conditions. Ten clusters showed specifically expression changes under UV-B, related to phosphoylation kinases, proteolysis functions and pathogen related responses. Ten clusters were specifically under cold stress conditions, three clusters showed an up regulated expression profiles, related with ethylene signaling response. The remaining clusters present a down regulated expression profiles without enrichments. Finally seven clusters shows differential expression related with salt stress response. Five of them including an up regulated expression, involving the abscisic acid pathway, oxidative stress response and secondary metabolic processes. Additionally, two clusters showed a down regulated expression profiles enriching plant development structures. This analysis allows us to identify particular genes and functional process related to *A. thaliana* under different stress conditions in order to identify new markers to characterize differential abiotic stress response. *Partially funded by D11-2004 project, Dirección de Investigación, Universidad de La Frontera.*

5.Determination of the binding site of phosphatidylinositol-bisphosphate (PIP2) to transient receptor potential channels (TRP). Poblete, H.[1], Oyarzun, I.[2], González-Nilo, D.[1,2], Latorre, R.[2]. Centro de Bioinformática y Simulación Molecular, Universidad de Talca, 2 Norte 685, Casilla 721, Talca, Chile. [1], Centro Interdisciplinario de Neurociencias de Valparaíso, Facultad de Ciencias, Universidad de Valparaíso, 287 Gran Bretaña, Valparaíso 2360102, Chile.[2].
Transient Receptor Potential channels (TRP) encompass a large family that is sub classified in 8 distinct subfamilies, namely TRPC, TRPM, TRPV, TRPA, TRPP, TRPML, the TRPN subfamily found in invertebrates and the distant TRPY subfamily present in yeast. Those receptors play a pivotal role in the transduction of different somatosensory modalities in mammals, including thermosensation, pheromone reception, regulation of the vascular tone, nociception and pain. In some members of TRP family, the channel activity is controlled by the second messenger fosfatidilinositol-4,5-bifosfato (PIP2). In particular, the TRPV1 is a polymodal receptor activated by heat (high temperatures). This channel has in the proximal region of the C-terminal a TRP domain and a pocket called ?TRP box? that is highly conserved in the subgroup called Thermo-TRPs. It has been suggested that this motif could be involve in the activation of these channels by lipids such as PIP2. In order to understand the gaiting process, it has been generated a homology model of the receptor, the model is the high complexity because requires the assembly of 4 different domains. This model has allowed the identification and characterization of key residues in the process of recognition and channel activation by PIP2. The experimental data have validated the homology model and have contributed to the determination of the structural motif that governs the mechanism of activation of TRPV1 channels. CINV-ICM.

6.Structural characterization and substrate affinity of two different isoforms of alcohol acyltransferase from *Cucumis melo* involved in ester biosynthesis. Galaz, S.[1], Morales-Quintana, L.[1], Moya-Leon, M.A.[1], Herrera, R.[1]. Instituto de Biología Vegetal y Biotecnología, Universidad de Talca[1].
Aroma is an important attribute of fruit quality, determined by a set of low molecular weight volatile compounds. In melon fruit (*Cucumis melo*) esters are profusely produced during its ripening, and synthesized through alcohol acyl transferases (AAT). Four different isoforms have been identified in melon. Interestingly, the ability to produce ester is different, being CmAAT3 a very active enzyme while CmAAT2 is not active. To clarify the enzymatic mechanism of CmAAT3 and CmAAT2, comparative modelling methodology was used to build a structural model of these enzymes. The models were validated and refined by molecular dynamics simulations. CmAAT3 has a similar structure to CmAAT2. CmAAT3 is composed of 17 beta sheets and 13 alpha helix, the active site is formed by the HTMSD segment located in a loop between the sheet 6 and the helix 2, while CmAAT2 is composed by 14 beta sheets and 11 alpha helix and the active site is formed by the HTMAD segment located in a loop between the sheet 10 and the helix 9. Using molecular docking simulation the interaction of each enzyme with different substrates (alcohols and acyl CoAs) was explored. CmAAT3 showed favourable affinity energies with different substrates, while unfavourable affinity energies were obtained for CmAAT2. An excellent coincidence was observed between docking studies and the AAT activity reported for these proteins. L.M.-Q. acknowledges CONICYT for a Doctoral fellowship. Research supported by Anillo ACT-41 project.

7.Deep-sequencing analysis of small RNAs in the *Xenopus tropicalis* gastrula. Almonacid, L.[1,2], Faunes, F.[3], Lee-Liu, D.[3,4], 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], Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile.[4].
The identification of different classes of small RNAs (miRNA, siRNA and piRNA) is one of the most important findings in the study of the transcriptome in the last two decades. In *Xenopus,* using deep sequencing technologies, different libraries of small RNAs (sRNAs) have been successfully prepared and characterized from oocytes, eggs, liver and skin. During *Xenopus* development, anterior-posterior and dorso-ventral patterning begins mainly at the gastrula stage, 10 hours after fertilization. Several miRNAs are expressed in gastrula stage embryos, although, detailed characterization of the pool of sRNAs of the *Xenopus* gastrula is not yet available. In order to gain information about sRNAs in this stage and to compare against other available sRNA libraries, we used Illumina sequencing technology to characterize the small RNAs in gastrula stage embryos of *X. tropicalis*. A total of 17,553,124 small RNA sequences were perfectly matched to the *X. tropicalis* genome. We used the Ensembl annotation to map small RNAs to genes and intergenic regions. The length distribution of our library suggested that piRNAs are the most abundant class at the gastrula. We identified putative loci of piRNA precursors in the genome and compared these loci to previous libraries. This is the first characterization of small RNAs in an embryonic tissue during *Xenopus* development. ACKNOWLEDGEMENTS This research was funded by grants from FONDECYT (1070357) and ICM (P09-016-F).

8.Dendritic nanoparticles (PAMAM) used as an optimal mechanism for transport and controlled drug delivery. Vergara-Jaque, A.[1], Monsalve, L.[1], Sandoval, C.[1,2], González-Nilo, D.[1,2]. Center for Bioinformatics and Molecular Simulation, Universidad de Talca, 2 Norte 685, Casilla 721, Talca, Chile. [1], Nanobiotechnology Division at University of Talca, Fraunhofer Chile Research Foundation - Center for Systems Biotechnology, FCR-CSB, Talca, Maule, Chile.[2].
Numerous efforts have been focused on the development of drug carrier systems able to enhance drug therapeutic efficacy. Polyamidoamine (PAMAM) dendrimers have been widely considered for pharmaceutical industry as an optimal mechanism for transport and controlled drug delivery. Properties such as biocompatibility, water solubility, versatility in modifying their functional groups, and responsiveness of their conformational properties to an aqueous environment become these macromolecules appropriate for such uses. Amine-terminated PAMAM dendrimers are able to solubilize different families of hydrophobic drugs, but the cationic charges on dendrimer surface may disturb the cell membrane. Therefore, acetylation is a convenient strategy to neutralize the peripheral amine groups and improve dendrimer biocompatibility1. The aim of this work is to analyze the structural properties that determine the encapsulation of drug-like molecules into PAMAM-G5 and PAMAM-G5-Acetylated dendrimers, using Dexamethasone 21-phosphate (Dp21) as a model drug. For this purpose, PAMAM dendrimers and Dp21 were parameterized using the new CGenFF force field. Then, full atomistic molecular dynamics simulations, in aqueous solutions at different pH conditions, were employed to position Dp21 into internal cavities of dendrimer and characterize the host-guest chemistry of acetylated dendrimer/Dp21 and cationic dendrimer/Dp21 complexes. Our results show that the orientation of Dp21 molecules in the dendimer cavities depends on the quaternization degree of tertiary amine groups of dendrimer and the protonation ratio of phosphate groups of Dp21. These results provide a new insight for the design and optimization of biocompatible dendrimer-based drug delivery systems. PBCT ACT/24.

9.Small molecules determination based in the Gβγ-GlyR interaction site. Cerda, F.[1], San Martín, L.[1], Martínez, J.[2], Jiménez, V.[3], Guzmán, L.[1]. Departamento de Fisiología, Universidad de Concepción, Concepción, Chile.[1], Departamento de Bioquímica y Biología Molecular, Universidad de Concepción, Concepción, Chile.[2], Departamento de Química Orgánica, Universidad de Concepción, Concepción, Chile.[3].

Recent findings indicate that Glycine Receptor (GlyR), a Ligand Gated Ion Channel (LGIC), is an effector of the Gβγ dimer. It has been demonstrated that the bindng region between Gβγ and GlyR is located in a motif of basic amino acids within the cytoplasmic domain (between transmembrane domain 3 and 4). In this works we have used this motif as a peptide (RQHc7). In electrophysiological assays this peptide was capable to interfere with the binding of Gβγ and GlyR. Moreover, bioinformatic tools have been used to determine, first the modeled structure of RQHc7. Then, using docking and molecular dynamics, the interaction site in Gβ was determined. These results indicate that three aspartic acids (186, 228 and 246) of Gβγ are the most important for this interaction. Finally, based in this interaction site, a virtual screening was performed with the small molecule library ZINC. Analyzing the affinity of the molecules to Gβ eighty high affinity small molecules were selected. These findings would be the initial steps for future researches in the pharmacological intervention of ethanol effects. This work was supported by Innova Bio-Bio Grant N° 10 CH S2 697 F11 and FONDECYT Grant 11080145.

10.Role of solvent channel in substrate selectivity of two different alcohol acyltransferase from climacteric fruit species. 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].

Tropical papaya (*Carica papaya*) and mountain papaya (Vasconcellea pubescens) fruit are characterized for their strong aroma. These compounds are different and dominated by esters, which are synthesized by the enzyme alcohol acyltransferases (AAT). A functional AAT gen has been previously identified in *V. pubescens* (VpAAT1), while a sequence coding for an AAT (CpAAT1) has been identified from *C. papaya*. Both genes showed 69.6% identity. Interestingly, the ability to produce ester is different, being VpAAT1 a very active enzyme towards the production of benzyl acetate while *C. papaya* is more active towards the production of methyl butanoate. The structural model of CpAAT1 protein was built by comparative modelling methodology. Conformational interaction between the protein and several ligands was explored by molecular docking. CpAAT1 structure consists of two domains connected by a large crossover loop, with a solvent channel in the center of the structure, having similar structure to VpAAT1. Even if they showed similar catalytic sites, the solvent channels are different in size and shape. Solvent channel of CpAAT1 is larger, which could explain their higher selectivity for large substrates. This fact could explain the differences in esters produced by these two fruits. Additionally, the most favourable predicted substrate orientation in CpAAT1was observed for methyl butanoate, showing a perfect coincidence between volatile abundance in the fruit and molecular docking analysis.L.M.-Q. acknowledges CONICYT for a Doctoral fellowship. Research supported by Anillo ACT-41 project.

11.Structural characterization of PhpAAT1 enzyme and its importance in ester production in *Physalis peruviana*. Carrasco-Orellana, C.[1], Morales-Quintana, L.[1], Zuñiga, R.[1], Moya-León, M.A.[1], Herrera, R.[1]. Instituto de Biología Vegetal y Biotecnología, Universidad de Talca.[1].

Goldenberry (*Physalis peruviana L.*) is a climacteric fruit with a pleasant aroma. The aroma of goldenberry fruit is mainly provided by esters, which are synthesized by the enzyme alcohol acyltransferase enzyme (AAT) employing different alcohols and acyl-CoAs as substrates. The *PhpAAT1* gene was recently cloned and the amino acid sequence was obtained. The gene sequence shared 24.5% similarity at the amino acid level to CmAAT4 isolated from *Cucumis melo*. In order to understand the mechanism of action of PhpAAT1, the enzyme three-dimensional structure was obtained by employing homology modelling tools and molecular dynamics studies. The model showed the segment HKLSD in the protein active site, being His154 located in helix 6 and Asp158 located in loop 13 between helices 6 and 7. This result was corroborated with 2 ns of molecular dynamics. To clarify the interaction of the protein with its substrates, different putative ligands were evaluated through molecular docking simulations. Results provided affinity energies and orientation data for each pair of ligands and the catalytic residues within the solvent channel. The most favourable predicted substrate orientation was observed for butyl acetate and butyl hexanoate, showing a correlation between ester production rate in the fruit and the affinity energies obtained by docking simulations. The protein model obtained allowed us to explain the differential biosynthesis rate of esters observed in goldenberry fruit.L.M.-Q. and R.Z. acknowledge CONICYT and Univeristy of Talca for their Doctoral fellowships, respectively. The research was supported by Anillo .

12.Dissecting functional domains on nucleotide sugar transporters. Moreno, I.[1], Moreno, A.[1], Donoso, M.[1], Moraga, C.[1], Nebel, J.[2], Orellana, A.[1]. FONDAP Centro de Regulación del Genoma, Núcleo Milenio en Biotecnología Celular Vegetal,Centro de Biotecnología Vegetal, Facultad de Ciencias Biológicas, Universidad Andrés Bello[1], Faculty of Computing, Information Systems & Mathematics, Kingston University, London[2].

Nucleotide sugar transporters (NSTs) are membrane proteins involved in the translocation of nucleotide sugars. Since these kind of proteins contains multiple helix domains and due to the lack of information about structures of related membrane proteins, the study of substrate specificity and transport mechanism becomes difficult to accomplish. To this end, we have performed a thorough analysis of NSTs on eukaryots, which reveal that certain groups of conserved aminoacids may explain the substrate difference between them. Also, we have identified the spliced variant of a human NST called hUGTrel1 (SLC35B1) that encompass the first two transmembrane domains linked by a loop. Transport assays in proteoliposomes suggest that this protein is able to transport UDP-Glucose, indicating that interacting domains involved in the binding and transport activity are present in this protein. Using this splicing variant and bioinformatics analysis, we propose that substrate specificity could be predicted analyzing these group of aminoacids. Futher analysis involving structure prediction using ab initio software will be discussed.Supported by FONDAP CRG-15090007; PCB-MN P02-009F; Fondecyt 1070379. AM and IM are supported by Programa de Formación de Capital Humano Avanzado CONICYT.

13.Kinetic folding simulations of the knotted ribbon-helix-helix (RHH) protein VirC2 using a simplified structured-based model. Ramírez, C.[1], Noel, J.K.[2], Baez, M.[1]. Department of Biology, Faculty of Sciences, University of Chile, Santiago, Chile.[1], Center for Theoretical Biological Physics, University of California, San Diego, La Jolla CA 92093-0374, USA[2].

The ribbon-helix-helix (RHH) superfamily of transcription factors presents a conserved structure of four helices in an open array of two hairpins. While typical RHH proteins are dimers with two RHH motifs (Arc and Met repressors) there are a few monomeric proteins (TraY, VirC2) comprising a duplicated RHH motif connected by a loop. Resolved structure of VirC2 shows that these monomeric proteins have a knotted topology. This intriguing topology leads to the question on how the knot is made during folding. To address this question, we performed kinetic folding simulations of VirC2 and Arc using a simplified coarse-grained structure-based protein model where each residue is represented by a single bead of unit mass whose coordinates are the same as the corresponding alpha carbon (Cα). A Cα contact map comprising only nonbonded interactions between residues pairs with at least one atom-atom contact in the native state are described by a Lennard-Jones potential. Our simulations show that the knot can be formed by accessing a slipknot conformation where the C-terminus is threaded through the loop connecting both RHH motifs or by flipping a loop around the N-terminus. While the latter resembles the Arc repressor folding mechanism, folding upon threading of the C-terminus corresponds to a kinetic intermediate where most of the native contacts are already made. These results suggest that duplication and knotting of two RHH motifs leads to a change in the folding pathway. (Partial support from Fondecyt 1090336 and NSF PHY-0822283).

14.The vitamin D receptor binds to nuclear matrix proteins in the presence and absence of vitamin D. Ruiz-Tagle, C.1, Nilo, R.1, Orellana, A.1, Montecino, M.1. FONDAP Center for Genome Regulation, Faculty of Biological Sciences, Universidad Andrés Bello, Santiago, Chile.1.

WITHDRAWN BY FIRST AUTHOR

The vitamin D receptor (VDR), member of the nuclear receptor superfamily, is a transcription factor that modulates the expression of genes in response to vitamin D, by binding to regulatory elements (VDRE) at target promoters. In osteoblastic cells, VDR modulates the expression of genes by interacting with transcriptional coactivators such as SRC-1 and DRIP205, as well as with regulatory factors of the basal transcription machinery. We have demonstrated that in osteoblasts VDR is associated with the nuclear matrix, a sub-nuclear structure that can mediate the spatial distribution of several nuclear proteins and that contributes to the efficient occurrence of several important nuclear processes. This presentation shows that nuclear matrix extracts isolated from osteoblastic cells treated with vitamin D, exhibit an enrichment of VDR, the transcriptional coactivator DRIP205 and the RNA polymerase II. On the other hand, we also show that there are distinct groups of proteins that interact with VDR in the presence and absence of vitamin D. In particular, through a comparative study by two-dimensional electrophoresis, we observe proteins that only interact with VDR in the absence of vitamin D, together with proteins that interact with the receptor only in the presence of the hormone. Together, these results indicate that interaction of VDR with the nuclear matrix in osteoblastic cells can be mediated through specific nuclear matrix components. In addition, our results support an experimental protocol to isolate and identify these proteins. FONDECYT 1095075, FONDAP 15090007, CONICYT Scholarship.

15.Protein secretion by *Penicillium purpurogenum* under catabolite repression. Eyzaguirre, J.[1], **Pérez, N.**[1], Navarrete, M.[1], Callegari, E.[2]. Universidad Andrés Bello[1], Universidad de Dakota del Sur, U.S.A.[2].

Penicillium purpurogenum is a fungus with the ability for adaptation to different nutritional environmental conditions by secreting a variety of enzymes. Several enzymes of the secretome of Penicillium purpurogenum have been studied when grown on different carbon sources. Previously we have compared proteomics profiles of enzymes secreted when sugar beet pulp and acetylated xylan are the carbon source, and under non-denaturing conditions we have detected several protein complexes involved in xylan degradation. To better understand the effect of the chemical composition of the carbon source on protein expression, we present in this work proteomic profiles of the *Penicillium purpurogenum* secretome grown under conditions of catabolic repression. Two different proteomics approaches: 2-DE and gel-free analysis by shotgun are utilized. We demonstrate the strong catabolite repression exerted by glucose which is reflected in the few proteins detected. Three proteins are identified from 2-DE and fourteen by shotgun. We also show the effect of fructose on the secretome composition: 34 proteins were identified by shotgun and 9 spots from 2-DE. The level of expression of these enzymes is not under the control of glucose or fructose and may be the key to understand the mechanism used by fungi to sense environmental conditions. Financial support: FONDECYT (Grant 1100084), UNAB (Grant DI-03-10/R).

16.Heterologous expression of phenylpropanoid pathway enzyme in bacteria. Guzmán, L.[1], **Robles, C.**[1], Quintanilla, I.[1], González , P.[1], Aguilar, F.[2]. Laboratorio de Química Biológica. Instituto de Química. Pontificia Universidad Católica de Valparaíso[1], Laboratorio de Fotoquímica. Instituto de Química. Pontificia Universidad Católica de Valparaíso[2].

The flavonoid/stilbenes are plant secondary metabolites from the phenylpropanoids pathway. The flavonoid/stilbene shown to have a broad spectrum of beneficial health effect such as: anti-cancer, anti-inflammatory, antimicrobial activity, among others. These biological activities have potential pharmaceutical use. The stilbene synthase (STS) play a key role in phenylpropanoid metabolism, catalyzing the production of flavonoid from coummaroyl-CoA and malonyl-CoA. Malonyl-CoA is synthesized by the action of acetyl-CoA carboxylase. However, the malonyl-CoA could be synthesized from malonic acid by malonyl-CoA synthetase (MCS) an enzyme found in bacteria of the genus Rhizobium. The aim of this study was to characterize the STS and MCS in a bacterial host. Each gene (mcs and sts) was amplified by PCR from genomic DNA (vitis vinifera and Rhizobium). PCR products were cloned into the pGEM?-T easy vector. Then, both gene was sub-cloned in tandem into an arabinose inducible shuttle expression vector (pBAD), generating pBSTSMCS. Gene expression of STS and MCS was induced with arabinose (2 g / L) from an E. coli (pBSTSMCS) growing in minimal medium by 60 min. The gene expression was analyzed by RT-PCR and proteins were analyzed by SDS-PAGE. The enzyme activity was analyzed in a coupled spectrophotometric enzyme assay in the presence of their substrates (malonate, ATP, Coenzyme A and 4-coumaroil-CoA). The purpose of this work is to optimize the expression of STS and MCS in a bacterial system. Supported by EcoTecnos Ltda and PUCV-VRIEA (project DII/PUCV 037/113-2010).

17.Multiplex real-time PCR for the specific detection of Piscirickettsia salmonis. Calquín, P.[1], Álvarez, C.[1], Valenzuela, K.[1], Cárcamo, J.G.[1], Avendaño-Herrera, R.[2], Yáñez, A.[1]. Instituto de Bioquímica y Microbiología, Universidad Austral de Chile[1], Laboratorio de Patología de Organismos Acuáticos y Biotecnología Acuícola, Universidad Andrés Bello[2].

Salmonid Rickettsial Septicemia (SRS) is a systemic disease caused by Piscirickettsia salmonis, responsible for high mortality in salmonids farming in Chile. Attempts to detect this pathogen using real-time PCR resulted in low specificity. Here, we developed and implemented a multiplex real-time PCR for the detection of P. salmonis. Specific primers were designed to target three different genes of P. salmonis strains that affect national farming. These oligonucleotides were combined in a single reaction tube. Primers specificity was evaluated using DNA samples from 31 different isolates of P. salmonis, from 6 different salmon pathogens, and from tissues of control and P. salmonis-infected fishes. The results showed that this multiplex real-time PCR allows rapid and specific detection of P. salmonis in tissue samples. The amplification patterns for individual genes were analyzed by agarose gels and cycle threshold (CT) of PCR reaction, showing positive amplification with control primers in all samples but the specific sets of primers from the kit only amplified samples with P salmonis DNA from 3,5 fg with CT 31,65 to 35 ng with CT 12,99. The size amplicons obtained were 150, 200 and 300pb with Tm values of 81.2°C, 80.5°C and 84°C respectively accordingly with the design. These data allow us to conclude that the designed format of real-time PCR is the faster and more precise technique to identify this pathogen. (INNOVA 07CN13PPT-256).

18.Characterization of proteins present in Sauvignon blanc wines and their role in protein haze formation. Lobos, F.[1], De Bruijn, J.[2], Martínez-Oyanedel, J.[1]. Laboratory of Molecular Biophysics, Department of Biochemistry and Molecular Biology, Faculty of Biological Sciences, Universidad de Concepción, Chile[1], Department of Agroindustry, Universidad de Concepción, Chillán, Chile[2].

Introduction Colloid formation in white wine due to protein denaturation and aggregation, also known as protein haze, is a problem that affects the wine industry, causing considerable economic loss. The current stabilization methodology has certain disadvantages, so alternatives are sought. This requires a complete understanding and characterization of the phenomenon in order to establish specific stabilization strategies. **Materials and Methods** Proteins from ultrafiltration fractions of Chilean Sauvignon blanc wine from Casablanca and Curicó valleys were characterized by biochemical techniques. Electrophoretic and chromatographic methods were used to determine molecular weights, isoelectric points and glycosilation states of the proteins present in these samples. **Results** Each valley and each fraction showed characteristic chromatographic and electrophoretic patterns. Proteins present in both wines have molecular weights located between 18 and 72 kDa, and isoelectric points between 3.2 and 8.8. Low molecular weight proteins (~25 kDa) were found in heat unstable fractions, while both 30-100 kDa and 100-300 kDa fractions have high molecular weight species (MW > 30 kDa), including glycoproteins. **Discussion** Low molecular weight proteins are involved to a greater degree in white wine haze formation, while glycoproteins would exert a protective effect against it. Particularly, a glycoprotein with low isoelectric point (3.2) and a molecular weight of ~65 kDa was found in a heat stable fraction. This protein was subsequently identified by mass spectrometry as a grape invertase. Altogether, these results show the varying roles of these proteins in haze formation. **Funding: FONDECYT 11085054**.

19.Encapsulation of proteins on alginate-chitosan mixtures. Silva, H.[1], Valenzuela, K.[1], Álvarez, C.[1], Sáez, M.[1], Pontigo, J.[1], Oliver, C.[1], Amthauer, R.[1], Yánez, A.[1]. Unversidad Austral de Chile[1].

The protein encapsulation is a technology used for immobilization, protection and controlled release of active principles for various applications ranging from medical treatment, sustainable agriculture, cosmetics, among others. Here, chitosan-alginate microcapsules were evaluated as a method of oral delivery of proteins as an oral vaccine. Physical characteristics, encapsulation efficiency, gastro-resistance, and release characteristics of these microcapsules in vitro and in vivo were investigated using colorants and bacterial proteins. Optimum physical factors were established for preparation of homogeneous, spherical, and smooth microcapsules. Colorants and proteins of *P. salmonis* bacterium were successful encapsulated with the polymers. The results showed that proteins of *P. salmonis* were released from microcapsules upon exposure to simulated intestinal fluid and not in the stomach. The stability of the proteins in gastric fluid was successful by encapsulation in chitosan-alginate microcapsules, and the absorption of proteins was stimulated. Moreover, microencapsulated proteins were significantly resistant to hydrolysis and were stable for 4 months. This technology allows us to produce several prototypes of oral vaccines against *P. Salmonis,* the most dangerous pathogen in the salmon industry. This work has support of: INNOVA CORFO-07CN13PPT256.

20.Evaluation of the effect on innate immunity of a new vaccine against *P.Salmonis.* Sáez, M.A.[1], Espinoza , C.[2,3], Pontigo, J.P.[1], Valenzuela, K.[1], Silva, H.[1], Troncoso, J.[3], Romero, A.[1], Yáñez, A.[1]. Universidad Austral de Chile[1], Universidad de Concepción[2], EWOS Innovation[3].

P.Salmonis is responsible of Salmonid Ricketsial Syndrome, this disease causes a important decrease in salmon production in our country and around the world. There are many efforts to produce new vaccines to prepare the salmon?s immune system against this pathogen. IL1 is a useful marker of innate immunity and is cytokine synthesized by macrophages, monocites and dendritics cells which participates in the maturation and proliferation of lymphocytes and activation of Natural Killer cells. In this work was analyzed the expression of this cytokine by Real Time PCR in headkidney samples of immunized salmons with a prototype of austral-vaccine against P.Salmonis. We observed an increment in the level expression of IL1 and between 3 at 7 days after the vaccination. A progressive decrease of basal level of IL1 was detected from day 30 to 45 post immunization. After 60 days post immunization, salmons were challenged with a DL80 using a reference strain of *P.Salmonis.* After 21 days the accumulative mortality in immunized and control salmons showed that in control group has and RPS near to 10%, and the vaccinated group 90% RPS. The results indicate that this new prototype of vaccines was able to induce a quick immune response confirming the use of IL1 as a marker of vaccine efficacy. Thos result support the possibility to determine the vaccine efficacy by following the correlation between cytokine response and protection conferred by immunization. This work was supported by INNOVA-CORFO 07CN13PPT-256.

21.The mitochondrial SncmtRNA and the modulation of the expression profile of cell cycle genes. Oliveira-Cruz, L.[1], Lyons, J.[2], Arraya, M.[1], Vidaurre, S.[3], Burzio, V.[3], Burzio, L.[3]. Andes Biotechnologies, Fundación Ciencia para la Vida[1], Andes Biotechnologies, University of California-San Francisco[2], Andes Biotechnologies, Fundación Ciencia para la Vida, Universidad Andrés Bello[3].

We characterized a family of noncoding mitochondrial RNAs (ncmtRNAs) in human and mouse, which contain an inverted repeat (IR) attached to the 5 'end of the sense (SncmtRNA) or antisense (ASncmtRNA) 16S mitochondrial rRNA. These transcripts form stem-loop structures and their expression profile differs depending on proliferative status; normal proliferating cells express both the S and ASncmtRNA, tumor cells express only the S and down-regulate the AS transcript and resting cells express neither. Knockdown of the ASncmtRNA in tumor cells using antisense technology (ASO) induces a decrease in proliferative index, followed by apoptotic cell death. To determine changes in cell cycle gene expression triggered by knockdown of the ASncmtRNA we carried out cell cycle qPCRarray (Sabioscience-Qiagen) containing 84 cell cycle-related genes, using RNA from H292 cells (lung carcinoma) transfected with an ASO against ASncmtRNA and an unrelated ASO as control. After 3 independent experiments, we found six upregulated genes, classified into three major categories: tumor suppressor, apoptosis-related and proteasomal degradation. We also found 13 downregulated genes, mostly involved in the G1/S transition. In light of these results, we sought to determine the effects of overexpression of the ncmtRNAs on the cell cycle. As a first step we have cloned SncmtRNA into a mammalian expression vector and transfected HeLa cells with the construct. Overexpression of SncmtRNA was confirmed by qPCR. This model will help us decipher the role of these transcripts in cell homeostasis. Fondecyt 1110835.

22.LKB1 kinase is required for RHEB GTPase to activate AMP-activated protein kinase. Armijo, M.[1], Pincheira, R.[1], Castro, A.[1]. Laboratorio de Transducción de Señales y Cáncer. Departamento de Bioquímica y Biología Molecular. Facultad de Ciencias Biológicas. Universidad de Concepción.[1].

The small GTPase Rheb activates the mammalian target of rapamycin-complex 1 (mTORC1), which promotes cell growth in response to growth factors, energy levels and nutrients availability, and is implicated in cancer cell metabolism. AMP-activated protein kinase (AMPK) is a sensor of intracellular energy levels and negatively regulates Rheb/mTORC1 pathway. In response to metabolic stress, AMPK phosphorylates and activates the TSC2 tumor suppressor, a GTPase-activating protein (GAP), which inhibits Rheb and thereby mTORC1. Interestingly, we recently discovered a mTORC1-independet Rheb-to-AMPK feedback activation mechanism. However, how Rheb activates AMPK remains elusive. Since full activation of AMPK depends on AMP levels and phosphorylation by the LKB1 kinase, we propose that Rheb interacts and activates LKB1 thereby elevating AMPK activity. To corroborate this hypothesis, we used a LKB1-null cell model (HeLa cells) to study activation of AMPK mediated by Rheb. We overexpressed Rheb in these cells and then analyzed by western blot the activation of AMPK. As expected, Rheb was unable to regulate AMPK activity in the absence of LKB1 expression. By coimmunoprecipitation assays, we demonstrated that Rheb interacts with endogenous LKB1 in HEK293T cells. Further studies are needed to determine the nature of this interaction and its physiological and pathological relevance. We expect to demonstrate that LKB1 is a novel Rheb effector that mediates Rheb's effects on AMPK and cellular energy homeostasis. DIUC 210.037.011-1.0; FONDECYT 1110821.

23.Silencing of tumor gene *WIF1* in hereditary triple negative breast cancer. Garrido, G.[1], Alvarez, C.[1], Tapia, T.[1], Cornejo, V.[2], Fernández, W.[2], Camus, M.[3], Alvarez, M.[4], Carvallo, P.[1]. Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile[1], Unidad de Anatomía Patológica, Hospital San Borja Arriarán, Santiago, Chile[2], Centro de Cáncer, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile[3], Clínica Las Condes, Santiago, Chile[4].

Triple negative breast cancer tumors (TNBC) are characterized by the lack of ER, PR and HER2, for which there is no specific treatment. TBNC are of high histological grade and have an aggressive clinical behavior. During breast cancer development, tumor suppressor genes can be silenced by different genetic, genomic and epigenetic mechanisms. In several cancer subtypes, including sporadic breast cancer, it has been described the silencing of tumor suppressor gene *WIF1* by hypermethylation of its promoter. Previously, using array CGH we found the deletion of the region of *WIF1* (12q14.3) in a group of hereditary breast cancer tumors, suggesting the involvement of this gene in hereditary breast cancer. Based on this information, we analyzed 30 formalin-fixed paraffin embedded (FFPE) tumor from woman with hereditary TNBC. DNA extraction was carried out by proteinase K and chemically modified with sodium bisulfite. Methylation analysis was done using a methylation specific PCR (MS-PCR) with primers for methylated and unmethylated status. We evaluated the expression of WIF1 protein through immunohistochemistry. The methylation analysis of hereditary TNBC tumors revealed high methylation frequency for *WIF1* (66%). In agreement, the methylated tumors showed moderate (64%) or weak (21%) WIF1 protein expression. Our results suggest that hypermethylation is an important mechanism for silencing WIF1 in hereditary TBNC. FONDECYT 1080595.

24.Knockdown of non-coding mitochondrial RNAs with antisense oligonucleotides induces changes in cell cycle-related proteins in normal and tumor cells. Fitzpatrick, C.[1,2,3], Vidaurre, S.[1,2,3], Oliveira-Cruz, L.[1,2,3], Briones, M.[1,2,3], Burzio, V.[1,2,3], Villegas, J.[1,2,3,4], Burzio, L.[1,2,3,4]. Fundación Ciencia para la Vida[1], Andes Biotechnologies SA[2], Universidad Andrés Bello[3], GrupoBios SA[4].

The novel family of non-coding mitochondrial RNAs (ncmtRNAs) displays a differential expression pattern according to proliferative status. In both cultured cells and tissue samples, normal proliferating cells express the sense (SncmtRNA) and antisense ncmtRNAs (ASncmtRNA). In contrast, tumor cells express the sense transcript and down-regulate de ASncmtRNAs, while normal resting cells express very low levels of both transcripts. Treatment with oligonucleotides (ODNs) directed against the ASncmtRNA induces massive and selective death of tumor cells by apoptosis, whereas viability of normal cells is unaffected by the same treatment (S. Vidaurre, unpublished results). Tumor cell death is preceded by a decrease in proliferative index, as shown by diminished incorporation of the BrdU analogue, EdU (S. Vidaurre and V. Burzio, unpublished results). Here we show that this change in the cell cycle is correlated with a decrease in CyclinD1, PCNA and E2F1 proteins. Normal mouse myoblasts (C2C12), which are resistant to death induced by knock-down of the ASncmtRNA, also suffer changes in the levels of CyclinD1 and PCNA, but when treated with ODNs against the SncmtRNA. In the case of normal cells, the decrease in proliferative index is reversible. Interestingly, preliminary determination of mRNA levels does not show a significant reduction that would explain the decrease in protein levels, (L. Oliveira-Cruz, unpublished results), suggesting that knock-down of ASncmtRNA exerts its effects on these genes by a post-transcriptional mechanism. This work was supported by PFB-16 and Fondecyt 1110835 (CONICYT).

25.Genomic aberrations and BRCA1 silencing in hereditary triple negative breast tumors in Chilean women. **Tapia, T.**[1], Sanchez, A.[1], Alvarez, C.[1], Cornejo, V.[2], Fernández, W.[2], Cruz, A.[3], Segovia, L.[3], Alvarez, M.[4], Camus, M.[5], Carvallo, P.[1]. Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile[1], Hospital San Borja Arriarán, Santiago, Chile[2], Hospital Barros Luco, Santiago, Chile[3], Clínica Las Condes, Santiago, Chile[4], Centro del Cáncer, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile [5].

Among breast cancer tumors, a group named as triple negative breast cancer (TNBC) does not express hormone receptors and HER2 (ER-, PR-, HER2-). For these tumors no specific treatment has been developed yet. It has been described that about 60-80% of TNBC tumors belong to the basal-like molecular subtype, constituting a biological and clinical distinct subgroup. To determine the basal-like subtype we evaluated the expression of Cytokeratin 5, 14 and 8/18 (CK5, CK14, CK8/18) marker in 41 hereditary TNBC tumors. Our results revealed that a 61% (25/41) of the TNBC tumors belong to the basal-like subtype, according to the expression of CK5 or/and CK14. Additionally, we analyzed the methylation status of the BRCA1 promoter and its correspondent protein expression in 41 TNBC tumors. A 68% (28/41) of the tumors had BRCA1 promoter hypermethylation. Of all tumors a 22% (9/41) had absent or reduced nuclear expression of BRCA1. Interestingly, we found a 63% (26/41) of the TNBC tumors with a cytoplasmic expression of BRCA1. Finally, we also analyzed through array-CGH, 27 hereditary TNBC tumors to identify common genomic aberrations between these tumors. The array-CGH analysis revealed the gain of the genomic regions 6p11.2, 16p13.3 and 17q12-21.32 and the deletion of the regions 1q21.1, 18q22.1-q22.3 and 19q13.41 in more than 20% of the TNBC tumors. Interestingly, the region deleted 1q21.1 was found in 68% of the hereditary TNBC tumors. Financed by FONDECYT 1080595 and CONICYT 24091058.

26.Effect of Temodal on the expression level of Major Vault Protein (MVP) in a Glioblastoma Multiforme cell line. **Calderón, F.**[1], Barrientos, C.[1], Manriquez, R.[1], Quezada, C.[1], Yáñez, A.[1], Cárcamo, J.[1]. Universidad Austral de Chile.[1].

The drug Temodal (temozolomide), an oral alkylating agent, is frequently utilized altogether withradiotherapy in the glioblastoma multiforme treatment. Like in other types of cancer, this tumor cellscan present a resistance mechanisms, through changes in the expression levels of several proteinsin response to the drug; as is the case of ABC protein family and MVP. MVP is a 110 kDa proteinand major component of the ribonucleoprotein particle Vault, probably involved in the movement ofdrugs from the nuclei to the cytosol, resulting in poor cytotoxic effect. There is not published dataabout effect of Temodal on the MVP expression levels in glioblastoma cells. We studied the effect of different concentrations of Temodal on the viability and expression levels ofMVP in glioblastoma- derivated cell line T98 through RT- PCR, western blot, immunofluorescenceand MTT assay.We found that, at 100 uM of Temodal, the level of the MVP RNAm and its proteinremain unchanged, while they are up regulated when the cells are treated with a concentration six-fold higher than this concentration. Under the experimental conditions used, it was notpossible to establish changes of the expression levels of MVP or in its subcellular localization byimmunofluorescence assays.The expression increase of MVP in response to the Temodal incubation, suggests a mechanismMVP- mediated that could play a significant role in the poor response observed in the treatment ofglioblastoma multiforme by this drug.

27.Detection of genomic rearrangements in the MLH1 and MSH2 genes in families with Hereditary Non Poliposis Colorrectal Cancer. **Orellana, P.**[1,2], Alvarez, K.[1,2], Hurtado, C.[1], Church, J.[3], Lopez-Kostner, F.[1], Carvallo, P.[2]. Laboratorio Oncología y Genética Molecular, Unidad de Coloproctología, Clínica las Condes[1], Departamento Biología Celular y Molecular, Facultad Ciencias Biológicas, Pontificia Universidad Católica de Chile[2], Cleveland Clinic Foundation, USA[3].

Approximately 5% of total cases of colorectal cancer correspond to Hereditary Non Polyposis Colorectal Cancer (HNPCC), caused by germline mutations in the DNA mismatch repair genes MLH1 and MSH2. MLH1 contains 19 exons spanning 100kb on chromosome 3p22.2. MSH2 contains 16 exons covering 73kb on chromosome 2p21. Different strategies are being used for detecting mutations in these genes. Our aim was the detection of genomic rearrangements (deletion/duplication) through Multiplex Ligation-Dependent Probe Amplification (MLPA) analysis. In this study, we analyzed 26 HNPCC families, non carriers for point mutations in these genes. We identified three different deletions in MLH1, in five families, and one deletion in MSH2. All these deletions affect only one allele and segregate with cancer in all families. MLH1 deletions involve: exon 1 in two families, exon 19 in two families, and exons 14 and 15 in one family. Exon2 deletion was detected in MSH2. This deletion was confirmed by RT-PCR in the MSH2 mRNA, revealing a fusion of exons 1 and 3. The mRNA sequence shows a frameshift and a premature stop codon. Deletion of exon 1, in MLH1, includes also the promoter, suggesting that this allele is not expressed. Deletion of exon 19 lead into the loss of the last 55 aminoacids, region necessary for interaction of MLH1 with PMS2. Finally, deletion of exons 14 and 15 lead into the loss of an important part of the enzyme, consisting in a deleterious mutation. Financed: Cleveland Clinic Foundation.

28.P53 tumor suppressor regulates the expression of the transcription factor SALL2 in response to genotoxic agents. **Escobar, D.**[1], Castro, A.[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 family of transcription factors, which play important roles in development. Clinical evidence relates Sall2 to cancer development. However, how Sall2 is involved in cancer has not been established yet.The p53 gene is the most frequently mutated gene in human cancers, about 50% of which have loss of p53 or express an inactive p53 mutant. Our studies show that Sall2 has transcriptional repressor activity in different cell lines. This activity is diminished in the presence of the p53 tumor suppressor. Overexpression of wild type p53, but not p53 mutants, decreases Sall2 levels suggesting that in cancers, where p53 is absent or mutated, Sall2 is deregulated. To determine the functional relationship between p53 and Sall2, we used a knock-in murine model that allows the induction of p53. Studies in primary fibroblasts derived from these mice show that p53 activation by genotoxic agents like doxorubicin, causes a decrease in both, Sall2 protein and mRNA levels. This effect does not occur in the absence of functional p53 protein. These results identify Sall2 as a novel downstream target of the p53 tumor suppressor protein and suggest that Sall2 and p53 may have an antagonistic role. We are further investigating Sall2 cellular function and its relationship with the p53 tumor suppressor to understand how Sall2 could contribute to cancer in general.FONDECYT Project 1110821.

29.The SALL2 transcription factor plays a role in cell proliferation. Sánchez, M.F.[1], Catro, A.[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].

Regulation of gene expression by transcription factors is one of the most important mechanisms for normal cellular function. Thus, it is not surprising that alteration of transcription factors has now been established as a frequent cause of tumorigenesis and neoplastic progression in humans. Sall2 is a poorly characterized transcription factor; its normal functions and the identity of the genes that it activates are largely unknown. Interestingly, Sall2 is deregulated in various cancers, which suggests it could play a role in cancer. We previously identified Sall2 as a novel interacting protein of neurotrophin receptors and showed that it plays a role in neuronal function, which does not necessarily explain why Sall2 could be deregulated in cancer.In this study, we examined the expression profile of Sall2 during the cell cycle, and its role in cell cycle progression. We found that Sall2 mRNA expression oscillates during the cell cycle with a peak of expression in S phase of primary mouse embryo fibroblast. Fibroblast derived from a Sall2 knockout mice show an increase in cell proliferation rate, and changes in the cell cycle distribution. Together, our result suggests a suppressive role for Sall2 in cell proliferation, which is in agreement with its putative role as tumor suppressor. We are further investigating Sall2 cellular function and its involvement with the cell cycle.FONDECYT Project 1110821.

30.M-RAS induces gen expression through a non canonic pathway in MCF-7 breast cancer cell. Campos, T.[1], Armijo, M.[1], Rivera, A.[1], Pincheira, R.[1], Castro, A.[1]. Laboratorio de Transducción de Señales y Cáncer. Departamento de Bioquímica y Biología Molecular. Facultad de Ciencias Biológicas. Universidad de Concepción.[1].

Ras proteins (H-, K- and N-Ras) are small GTPases that act as molecular switches that alternate between active GTP-bound and inactive GDP-bound states. These proteins are capable of regulating a wide variety of biological processes, including cell growth, differentiation and apoptosis. Constitutively activated mutants of Ras promote cellular transformation, and are found in approximately 30% of human cancers. M-Ras is a Ras-related GTPase involved in cell transformation and differentiation, but its mechanism of action and contribution to human malignancies are unknown. To determine which signal transduction pathways are important for the function of M-Ras, mutants were generated that differ in their ability to interact with different effectors. We demonstrated that the signaling events activated by M-Ras diverge among different human epithelial cell lines. We found that M-Ras induces MEK/ERK-dependent and -independent Elk1 activation in human MCF-7 breast cancer cell. This activity correlated with Ral GTPase/JNK activation and detectable endogenous cell-specific expression of Rlf, a guanine nucleotide exchange factor for Ral. In addition, constitutive activation of M-Ras overcame the estrogen-dependent growth of MCF-7 cells. Thus, our studies demonstrate that M-Ras signaling activity differs between human cells, highlighting the importance of defining Ras protein signaling within each cell type and cellular context, especially when designing treatments for Ras-induced cancer. DIUC 210.037.011-1.0; FONDECYT 1110821.

31.Cathepsin L effect on cell cycle in Caco-2 cells. Pérez, V.[1], Flaig, D.[1], Hermosilla, V.[1], Arrey, V.[1], Iribarren, C.[1], Leonardi, M.[1], Puchi, M.[1], Morin, V.[1]. Department of Biochemistry and Molecular Biology, Faculty of Biological Sciences, Universidad de Concepción. Chile.[1].

A variant of cathepsin L (SpH-protease) described in colon cancer cells has a differential localization in cell cycle stages, observing that during mitosis this protease colocalizes with alpha-tubulin and in the S phase it localizes preferentially in the nucleus. To establish if SpH-protease is essential during the cell cycle of Caco-2 cells, the cathepsin L inhibition was determined. Firstly, the duration of the cycle of the Caco-2 cells was determined. In this respect, the S phase has a duration of 8 hours, as the bromodeoxyuridine essays show, whereas mitosis lasts 5 hours, according to DAPI staining. Finally, phases G1 and G2 have a duration of 2 to 3 hours. Therefore, the cycle of Caco-2 cells have a duration of 18 hours. Secondly, the effect of cathepsin L inhibition on the cell cycle of Caco-2 cells by immunofluorescence was analyzed. The results obtained indicate that cathepsin L inhibition causes a 4 hours delay at the end of the mitosis, whereas the cathepsin L inhibition during the G1 phase leads a delay during the S phase entry in 4 hours. Additionally the SpH-protease expression in cell cycle stages by RT-PCR was analyzed, showing that this protease is expressed in all phases of the cycle. In conclusion, the localization of SpH-protease in the cycle phases and the effect of cathepsin L inhibition, make us claim that this protease could correspond to cathepsin L involved in the cell cycle of Caco-2 cells. FONDECYT 11070067.

32.Computer assisted design and chemical synthesis of apamin mimetic peptides to study their interaction with the human protein BIRC-5 expressed in *E. coli*. Carrasco, V.[1], Guzmán, L.[1], Muñoz, E.[2], Guzmán, F.[3], Aguilar, L.[1]. Instituto de Química. Pontificia Universidad Católica de Valparaíso. Avenida Universidad #330. Campus Curauma, Valparaíso[1], Instituto de Física. Pontificia Universidad Católica de Valparaíso. Avenida Universidad #330. Campus Curauma, Valparaíso[2], Núcleo Biotecnología Curauma. Pontificia Universidad Católica de Valparaíso. Avenida Universidad #330. Campus Curauma, Valparaíso[3].

Finding specific inhibitors for proteins involved in the cancer cells proliferation is of vital importance for the drug development of new cancer therapies. Survivin (BIRC-5) protein is a member of inhibitors of apoptosis proteins family (IAPs). Survivin binds to Smac/DIABLO preventing this protein to interact with other IAPs and it also inhibits apoptosis. In this study we investigate the possibility of generating peptides that mimic the N-terminus of Smac/DIABLO. These peptides were designed using Apamin as a template. The advantage of Apamin as a template is the conservation of alpha-helix conformation with partial modification of its amino acid structure. A range of alpha-helix peptides were designed and synthesized that might be able to interact with Survivin. The peptides were designed through molecular bioinformatics using sequence homology modeling. The preparation of peptides was done by solid phase peptide synthesis (SPPS) and characterized by MALDI-TOF and CD. In order to obtain Survivin, total RNA was extracted from carcinoma gastric cell line (MKN45). The survivin gene was amplified by RT-PCR and cloned in the expression vector *pRSETB*. The recombinant survivin protein was expressed from *E. coli BL21* (DE3) by IPTG induction and purified by chromatography on metal-chelating resin. Once purified, IAP was characterized according to their molecular weight by SDS-PAGE. Changes in the structure and dynamics of Survivin were characterized by fluorescence spectroscopy. **Acknowledgements**: This work was supported by Dirección de Investigación e Innovación of the VRIEA; project DII/PUCV 037.359/2011.

33.CXCR3 and their spliced variants as tumour marker for Papillary Thyroid Cancer? Véliz, L.[1,2], Vargas, S.[1], Bohmwal, K.[3], Catalán , T.[2], Kalergis, A.[2], Riedel, C.[3], González, H.[1]. Departamento de Cirugía Oncológica, Pontificia Universidad Católica de Chile, Santiago, Chile.[1], Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile.[2], Facultad de Ciencias Biológicas, Universidad Andrés Bello, Santiago, Chile.[3].

The chemokine receptor CXCR3 and its splicing variants (CXCR3A and CXCR3B) has been involved in cellular proliferation, chemotaxis and in angiostatic effect. Recently, we demonstrated that CCR7 and CXCR4, are upregulated in pacients with papillary thyroid carcinoma (PTC) suggesting that chemokine receptors are good markers for PTC. The purpose of this study was to analyze the expression of CXCR3 and its spliced variants in PTC and evaluate their use as biomarkers in thyroid cancer.Samples of PTC and matched non-malignant tissue (contralateral thyroid lobe) were obtained from 30 patients. Messenger RNA and protein expression was evaluated in CXCR3 and its splicing variants CXCR3A and CXCR3B. Levels of mRNA were analyzed by Real Time PCR and protein expression was assessed by immunohistochemistry.Our qRT-PCR results showed significant decreased levels of CXCR3 and both its spliced variants in tumor samples compared to non-malignant thyroid tissues. On the contrary, immunohistochemistry revealed almost a fivefold increased of CXCR3 protein expression in cancer cells compared to the thyroid epithelium of matched non-malignant tissue. We have detected very significant changes of mRNA and protein expression of CXCR3 in PTC compared to non-malignant thyroid tissue, and therefore, is a potential candidate as a tumor biomarker in PTC. The mechanisms that determine the opposite changes of mRNA and protein expression of CXCR3 are under investigation. Biomedical Research Consortium.

34.Effect of phorbol esters over nuclear cathepsin L in colon cancer cells. Hermosilla, V.[1], Pérez, V.[1], Flaig, D.[1], Iribarren, C.[1], Leonardi, M.[1], Puchi, M.[1], Morin, V.[1]. Department of Biochemistry and Molecular Biology, Faculty of Biological Sciences, Universidad de Concepción.[1].

The study of molecular mechanisms taking place in cancer development involves the use of tumors promoters compounds which promote cell proliferation, being phorbol 12-myristate 13-acetate or PMA one of the most used. The effectors of this compound at a cellular level correspond to the kinase family Protein kinase C or PKC, which participates in several signal transduction pathways that regulate events such as proliferation, survival and cell differentiation. Deregulation of these signaling pathways in presence of PMA leads to activation or inactivation of other proteins such as cathepsin L, protease which is involved in the terminal degradation of proteins within the lysosomes, being also important for cell proliferation through the activation of CDP/Cux transcription factor in the nucleus. In order to study the effect that PMA has over cathepsin L at a nuclear level, colorectal cancer cells Caco-2 were treated with this compound and cell proliferation was evaluated through incorporation of 5-bromo-2-deoxyuridine. In parallel, expression and location of Catepsina L was observed through western Blot and confocal microscopy, and its proteolytical activity was assessed by cleavage of the fluorogenic substrate Z-Phe-Arg-MCA. It has been proved that in this cell line PMA produces an increase in the percentage of mitotic cells, accompanied by an increase in the level of cathepsin L and changes in cell morphology. These results provide new data for the understanding of PKC deregulation in carcinogenesis. FONDECYT 11070067.

35.Docking Studies of Coumarins Derivatives as lipoxygenase Inhibitors. Muñoz, A.[1], García-Beltrán, O.[2], Mascayano, C.[1], Nuñez, M.T.[3,4], Cassels, B.K.[2,4], Fierro, A.[1,5]. 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 Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile.[3], Instituto Milenio de Dinámica Celular y Biotecnología, Santiago, Chile.[4], Instituto Milenio de Dinámica Celular y Biotecnología, Santiago, Chile[5]. Sponsored by F. Gonzalez Nilo.

The biological properties of lipoxygenases (LO's) 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-hLO), breast cancer and psoriasis (12-hLO) and colorectal cancer and atheriosclerosis (15-hLO) have been related with isoforms of LO's. 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 lipoxygenase inhibitors.In this research a systematic study of coumarins derivatives using molecular simulation methodologies was done in order to understand the binding modes and to obtain mechanistic insights. **Acknowledgments**Financial support from DICYT-USACH project # 021041MC, Fondecyt 11085002, PDA-23 and ICM-FIC P05-001-F is gratefully acknowledged.

36.B-Metilated phenethylamines: the main interactions with monoamine oxidases and monoamine transporters. Zamora, R.[1], Rebolledo, M.[2], Fierro, A.[1,3]. Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile. [1], Facultad de Ciencias, Universidad de Chile, Santiago, Chile.[2], Instituto Milenio de Dinámica Celular y Biotecnología, Santiago, Chile.[3] Sponsored by M. Imara.

Monoamine oxidases and monoamine transporters are macromolecules that are implicated in the neurotransmitters level control in the synaptic space. The structural similarities that exist between phenethylamine derivatives and monoaminergic *neurotransmitters such as serotonin, dopamine, and norepinephrine* explains the effects of these derivatives in many processes that are normally controlled by these neurotransmitters. Amphetamine derivatives (phenethylamines alpha methylated) have been described as monoamine oxidase inhibitors and as neurotransmitter releasing agents [1]. In order to contribute at a systematic study of phenethylamine derivatives in MAO and serotonin transporters a series of beta-methylated phenethylamines were investigated using experimental and computational methodologies. The results suggest that the electronic effect of the para-substituent in the aromatic moiety associated with the conformational change on the alkylamine chain is responsible for the affinity of these new ligands by monoaminergic proteins. [1] Nichols, D. E. Medicinal Chemistry and Structure-Activity Relationships Cho, A. K. a. S. D. S., Ed.; Academic Press: San Diego, CA, 1994; pp 3-41.Financial support from Fondecyt 11085002, PDA-23 and ICM-FIC P05-001-F.

37.Chalcones as monoamine oxidase inhibitors. Morales, N.[1], Moya-Alvarado, G.[1], Dahech-Levenberg, P.[1], Pérez, E.[2], Caroli Rezende, M.[3], Fierro, A.[3]. Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile[1], Facultad de Química, Pontificia Universidad Católica de Chile, Santiago, Chile.[2], Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile. [3] Sponsored by G. Zuñiga.

nChalcones are part of a variety compounds that display anticancer, anti-inflammatory, and antioxidant activities among others. Some chalcone derivatives have been studied as Monoamine Oxidase inhibitors (MAOI) and through molecular simulation new insights on the inhibition activity have been obtained [1].MAO exists in two isoforms, A and B. Their structures, functions and mechanisms have been the focus of many researchers to understand the role of these proteins. MAO inhibitors are used in the treatment of psychiatric and neurodegenerative diseases [2].In this work we studied in rat MAO A and B, the biological activity of some chalcone derivatives and then, the specifics interactions in the catalytic cavity were investigated using molecular simulation methodologies.References:[1] Franco Chimenti, Rossella Fioravanti, Adriana Bolasco, Paola Chimenti, Daniela Secci, Francesca Rossi, Matilde Yañez, Francisco Orallo, Francesco Ortuso, and Stefano Alcaro, J. Med. Chem.2009, 52,2818-2824.[2] Edmondson DE, Binda C, Wang J, Upadhyay AK, Mattevi A. Biochemistry. 2009, 48(20):4220-30.Financial support from Fondecyt 11085002, PDA-23 and ICM-FIC P05-001-F.

38.Specific interactions between mescalinoids and the serotonin trasnsporter as possible MDMA (ECTASY)-like derivatives: an *in silico* study. Dahech-Levenberg, P.[1], Guajardo, C.[1], Morales, N.[1], Moya-Alvarado, G.[1], Sáez-Briones, P.[2,3], Zapata-Torres, G.[3,4], Fierro, A.[3,5]. Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile[1], Facultad de Ciencias Médicas, Universidad de Santiago de Chile, Santiago, Chile.[2], Instituto Milenio de Dinámica Celular y Biotecnología, Santiago, Chile.[3], Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile.[4], Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile.[5] Sponsored by A. Moene.

The serotonin transporter (SERT) is a membrane spanning protein that uses Na+, K+, and Cl- gradients to allow the pre-synaptic neuron to reuptake its released neurotransmitter from the synaptic cleft. SERT is a major molecular target for MDMA, a unique psychoactive abused amphetamine with high therapeutic potential in the treatment of several neuropsychiatric disorders. The rational design of new ecstasy-like molecules is a mostly unexplored field in neuropharmacology. Mescalinoids are derived from the B-phenylethylamine mescaline, a naturally occurring phenylalkylamine hallucinogen contained in the cactus Lophophora willliamsii. Because some phenylalkylamines might share some aspects of the pharmacology of MDMA, we hypothesize that the rational modification of the mescaline template might generate new MDMA-like derivatives [1].In the present study, a prospective characterization of the interactions of a selected group of mescalinoids with SERT has been carried out using molecular simulation methodologies. A three-dimensional structure of SERT was obtained by homology modeling from the atomic-resolution X-ray crystal structure of the eubacterium *Aquifex aeolicus* leucine transporter (LeuT) [2]. Then, the main interactions to mescaline and for a series of brominated mescalinoids the molecular dockings were done. The results obtained indicate that Asp400 and Glu415 are key elements associated with the mescalinoid binding mode. References:[1] Shulgin, A.T. & Shulgin, A, Berkeley, CA, Transform Press, 1991.[2] Yamashita A., Singh S. K., Kawate T., Jin Y. and Gouaux E.; Nature, 437, 215-223, 2005.Financial support from DICYT-USACH 021001SB, Fondecyt 11085002, 1090037, PDA-23 and ICM-FIC P05-001-F.

39.Pharmacophore analysis of isoflavonoids as human lipoxygenase inhibitors. Espinosa, V.[1], Mascayano, C.[2], Sepulveda-Boza, S.[1], Holman, T.[3]. Escuela de Medicina, Facultad de Ciencias Médicas, Universidad de Santiago, Chile, Casilla 442,Correo 2 Santiago-Chile[1], Departamento de Ciencias del Ambiente, Facultad de Química y Biología USACH[2], Department of Chemistry and Biochemistry, University of California, Santa Cruz, California, 95064[3]. by F. Gonzalez Nilo.

The lipoxygenases (LO) are a family of non-heme iron-containing dioxygenases which catalyze the stereospecific insertion of molecular oxygen into arachidonic acid. Computational studies including energy optimization and low energy conformers generation were done on MOE 2009 for 16 compounds in 5 series (series 1-4 isoflavons-derivatives and 5 with isoflavans). The flexible alignment and consensus pharmacophore for series and active inhibitors against 5-hLO, platelet 12-hLO, reticulocyte 15-hLO-1 and prostate epithelial 15-HLO-2 were obtained. The pharmacophore analysis of serie 5 isoflavans, the best inhibitors of all lipoxygenases (IC50 lower than 40?M) showed that the C ring on this series are recognized as hydrophobic centroids instead the aromatic/hydrophobic feature identified on isoflavons, with a rigid structure and electrons on ? orbitals. The compound 5A presented a ten-times higher IC50 as 5-hLO inhibitor (7.1 ?M) compared with others in the serie, which presents a ?OH group in position 8 of A ring instead a ?CH3, the pharmacophore analysis indicates that methyl produced a hydrophobic centroid in the position replacing a donor/acceptor of H-bond Financial support from DICYT-USACH project # 021041MC Is gratefully acknowledged.

40.Enhanced ATP allosteric inhibition of phosphofructokinase-2 from *E. coli* induced by monovalent cations is unrelated to the dimer-tetramer transition induced by the nucleotide. Vallejos, G.[1], Peña, D.[1], Soto, C.[1], Baez, M.[1], Babul, J.[1]. Laboratorio de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Chile, Santiago[1].

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 the specific binding of monovalent cations such as K^+. Moreover, Pfk-2 functions as a homodimer and is able to form a tetramer in presence of MgATP, but this process seems not to be required for allosteric inhibition. However, it is unknown how specific binding of monovalent cations enhances the allosteric inhibition and its relationship with the dimer-tetramer transition induced by the nucleotide. In this work, the effect of K^+ on the allosteric inhibition of Pfk-2 was determined by kinetic assays and MgATP binding experiments measured by intrinsic fluorescence using the wild type enzyme and mutants unable to show the dimer-tetramer transition. It was found that K^+ enhances the allosteric inhibition both in the wild type Pfk-2 and in mutants unable to form tetramers, suggesting that the MgATP inhibition enhanced by K^+ is independent of the dimer-tetramer equilibrium. However, the apparent affinity for MgATP and sigmoidicity of the saturation curve is increased by the presence of K^+. Interestingly, mutants unable to form tetramers did not show significant variations either in the sigmoidicity or the apparent affinity by MgATP upon increasing the K^+ concentration. These results suggest that the dimer-tetramer transition induced by MgATP is favored in presence of K^+, but this situation cannot be extrapolated under catalytic conditions. Fondecyt 1090336.

41.Sequencing and expression in *Pichia pastoris* of the gene that codes for an alpha-glucuronidase from *Penicillium purpurogenum* and characterization of the heterologous enzyme. Rosa, L.[1], Ravanal, C.[1], Eyzaguirre, J.[1]. Universidad Andrés Bello[1].

Lignocellulose is the principal component of the plant cell wall. Renewable energy can be generated from its degradation for second generation biofuels production. Lignocellulose is composed of lignin, pectin, cellulose and hemicelulloses, xylan being the most abundant hemicellulose. For the complete hydrolysis of xylan (complex structure) numerous enzymes are required, acting on the xylan main chain or as debranching enzymes. Among the debranching enzymes is alpha-glucuronidase, the less studied hemicellulolityc enzyme. Alpha-glucuronidases liberate methyl glucuronic acid from glucuronoxylan, in which the uronic acid is linked to the non-reducing terminal xylopyranosyl residue. In our laboratory the fungus Penicillium purpurogenum is utilized as model organism for xylan degradation studies, since it secretes multiple hemicellulolityc enzymes. In the literature there are no reports of biochemical studies of alpha-glucuronidases from the genus Penicillium. The gene of interest has a size of 2523 pb and has no introns. This gene was heterologously expressed in Pichia pastoris GS115 for biochemical studies of the enzyme. The alpha-glucuronidase has a size of approximately 100 kDa. The enzyme acts on short xylooligosacharides but not directly on xylan. To generate these xylooligosacharides two endoxylanases from family GH 10 and 11 were used. The alpha-glucuronidase acts on xylan when the endoxylanase from family GH 10 is present. The alpha-glucuronidase from P. purpurogenum belongs to family GH 67. This enzyme has an optimal pH of 4.5-5.0 and an optimal temperature of 37 °C. Support: FONDECYT 1100084 and and UNAB DI-03-10/R.

42.Studies of an allosteric site and the trimer-trimer interface of *Bacillus caldovelox* arginase. García L., D.A.[1], Uribe, E.A.[1], Salgado, M.[1], Carvajal B., N.[1]. Laboratorio de Enzimología. Depto. Bioquímica y Biología Molecular. Facultad de Ciencias Biológicas. Universidad de Concepción.[1].

Arginase catalyzes the hydrolysis of L-arginine to L-ornithine and urea, and requires Mn^{2+} for catalytic activity. In our laboratory, we have corroborated the presence of an allosteric site in arginase from *B. caldovelox*, deduced from crystalographic data in the presence of L-arginine. As a consequence of mutations of residues that interacts with L-arginine in this site, there is a change in the observed kinetic pattern from Michaelian to cooperative. The bacterial enzyme is hexameric and in this study we have analyzed the structural and kinetic consequences of mutations in the trimer-trimer interface, specifically of Glu278, which establish a salt link with Arg278 from the adjacent trimer. Mutation of Glu278 to Ala, which compromises an interaction in this zone, resulted in trimeric and kinetically Michaelian species. This behavior changed to cooperative in the case of the double mutant E256A-E278A which also contains a mutation at the allosteric site. Analogous results were obtained for species resulting from mutations in Arg279. A bioinforamatic analysis revealed that mutations at the allosteric site have no effect on the trimer-trimer interface. Apart from demonstrating that trimers are catalytically active, we conclude that cooperativity results from interactions at the trimeric level and that residues Glu278 y Arg279 are critical for stabilization of the hexameric structure, but do not participate in the regulation of the cooperative properties exhibited by arginase from *B. caldovelox*.

43.Functional analysis of a LIM-domain of a rat brain agmatinase like protein. Vallejos, A.[1], Díaz, B.[1], Benitez, J.[1], Carvajal, N.[1], Uribe, E.[1]. Laboratorio de Enzimología, Depto. Bioquímica y Biología Celular, Fac. Cs. Biológicas, Universidad de Concepción[1].

Agmatinase catalyzes the hydrolysis of agmatine into putrescine and urea. Agmatine, a decarboxylated derivative of L-arginine, has been associated to several important biological processes in mammals, including neurotransmitter, anticonvulsant, antineurotoxic and antidepressant actions in the brain. We recently cloned and immunohistochemically detected a rat brain agmatinase-like protein (ALP), whose amino acid sequence greatly differs from other agmatinases and exhibits a LIM-domain in its C-terminal region. The protein was detected in the hypothalamic region, hippocampal astrocytes and neurons. Since truncated species, lacking the LIM-type domain exhibits a 10-fold increased K_{cat} and a 3-fold decreased K_M value for agmatine, our proposal has been that the domain functions as an autoinhibitory entity in ALP. To evaluate this suggestion, we have now cloned and expressed the isolated domain. As expected, the purified species contained 2 Zn^{2+} ions, which were are absent from the truncated enzyme. The isolated domain was inhibitory to truncated species, but not to the wild-type enzyme, and the inhibition was uncompetitive with a K_i value of 0,7μM. The results reinforce our postulated autoinhibitory role for the LIM-domain. Our suggestion is that this portion of ALP interacts with a yet unidentified brian protein and, in this manner, the inhibition of the enzyme is reverted. The LIM-domain appears, therefore, as playing a critical role in the regulation of agmatine concentrations in the brain. Grant DIUC 211.037.013-10.

44.Effect of Sodium Tungstate on the Localization of Insulin Receptor in Renal Tubule Cells of Diabetic Rats. Kairath, P.[1], Bertinat, R.[1], Soto, M.[1], San Martin, R.[1], Carpio, J.D.[1], Slebe, J.C.[1], Yañez, A.[1]. Universidad Austral de Chile[1].

The insulin receptor (InsR) is a disulfide-linked protein composed of two alfa and beta subunits present in the plasma membrane of target cells. The intrinsic subunits contain the tyrosine kinase domain that is important for glucose metabolism regulation. It has been reported that sodium tungstate has an effective normoglycemic efect, capable to regulate hepatic glucose metabolism thought insulin pathway. However the effect of this drug in diabetic nephropathy is unclear. Here, we characterized the effect of sodium tungstate treatment in the progression of diabetic nephropathy in relationship with the expression and localization of insulin receptor and biochemical parameters. The qRT-PCR analysis indicates a significant increment in the renal expression of the InsR gene in non-treated and tungstate-treated diabetic rats, respect to normal rats. By contrast, Western blot and inmunohistochemistry analysis showed a significant decrease in the protein expression in long term diabetic rats (4 month). Interestingly, the sodium tungstate treatments reestablish the level of protein expression of insulin receptor in kidneys of diabetic rats. In normal and diabetic rats InsR was localized in basolateral and apical plasma membrane of proximal and distal tubules. The treatment didn?t alter the localization of the receptor. The diabetic-induced change in the protein expression suggests an involvement of the InsR in the metabolic changes present during the progression of diabetic nephropathy and the mechanism used by tungstate in the reversion of this pathology (Fondecyt 1090694).

45.Glycogen accumulation and gluconeogenic enzymes expression in the kidney from IRS2 knockout mice. Bertinat, R.[1], Oliviera, J.[2], Kairath, P.[1], Slebe, J.C.[1], Gomis, R.[2], Yáñez, A.[1]. Universidad Austral de Chile[1], Hospital Clínic de Barcelona, Universitat de Barcelona[2].

Insulin receptor substrate (IRS) molecules are key mediators in insulin signaling pathway and play a central role in maintaining basic cellular functions, such as growth and metabolism. Four IRS proteins (1-4) has been described, but work with transgenic mice reveals that many insulin responses are mediated largely through IRS1 and IRS2. Although IRS2 appears to play a critical role in hepatic glucose metabolism, its participation in kidney metabolism is poorly understood yet. The aim of this study was to analyze glycogen and gluconeogenic enzyme expression in kidney and liver biopsies from control and IRS2 knockout (KO) mice. PAS staining revealed that glycogen accumulates in kidney tubules from both mice. Interestingly, KO mice treated with the normoglycemic insulin-mimetic agent sodium tungstate (NaW) showed larger glycogen stores in renal proximal convoluted tubules (RPCT) than straight tubules (RPST). Immunohistochemical analysis against the key gluconeogenic enzymes fructose 1,6-bisphosphatase (FBPase) and phosphoenolpyruvate carboxykinase (PEPCK) showed that their specific expression is maintained in all proximal tubules from control and KO mice. Surprisingly, NaW treatment only suppressed the expression of these enzymes in RPCT from IRS2 KO but not control mice. Glycogen accumulation, and FBPase and PEPCK expression and zonation were not altered in liver from control or KO mice, either treated or not with NaW. These results suggest that IRS2 participates in the NaW signaling pathway in kidney (FONDECyT 1090694).

46.GLP-1 promotes energetic metabolism in vascular smooth muscle cells. Morales, P.E.[1], Torres, G.[1], Michea, L.[2], Lavandero, S.[1,2], Chiong, M.[1]. Centro FONDAP Estudios Moleculares de la Célula, Facultad de Ciencias Químicas y Farmacéuticas/Facultad de Medicina, Universidad de Chile[1], ICBM, Facultad de Medicina, Universidad de Chile[2].

According to the National Survey of Health, nearly 1.2 million Chilean people are diabetics. Migration and proliferation of vascular smooth muscle cells (VSMC) are increased in these patients, promoting atherosclerosis development. The newest treatment for type 2 diabetes mellitus includes the use of incretin hormone analogs. The incretin glucagon like peptide 1 (GLP-1) facilitates glucose metabolism in adipocytes, skeletal and cardiac muscle. However, no information is available about effects of GLP-1 on VSMC. The aim of this study was to assess the action of GLP 1 on the glucose metabolism in the rat aortic cell line A7r5. Cells were exposed to GLP-1 (100 nM) for different times and glucose uptake was assayed using 3H-2-desoxyglucose while total ATP content and mitochondrial membrane potential were assayed by the luciferin/luciferase method and by JC1 and flow cytometry, respectively. GLP-1 increased glucose uptake after 1 h and this effect was prevented by the general inhibitor of glucose transporter cytochalasin B. Additionally, ATP production was augmented after GLP-1 stimulation. These responses may be mediated by a mitochondrial-related mechanism, given the increases in ATP and mitochondrial membrane potential. Together, these results show that GLP-1 promotes energetic metabolism in VSMC. FONDECYT 1110180 (MC); FONDAP 1501006 (SL, MC, LM), Becario Magíster CONICYT (PM), Becaria Doctorado CONICYT (GT).

47.Gibberellin biosynthesis in *Fusarium graminearum* complemented with the gibberellin biosynthesis genes from *Fusarium fujikoroi*, Amaya, M.I.[1], **Tudzynski, B.**[2], **Rojas, M.C.**[1]. Departamento de Química, Facultad de Ciencias, Universidad de Chile[1], Institut für Botanik, Westfälischen Wilhelms-Universität Münster[2].

Gibberellins (GAs) are diterpene phytohormones synthesized as secondary metabolites by *Fusarium* species that belong to the *Gibberella fujikuroi* species complex. Particularly *Fusarium fujikuroi* produces high levels of gibberellic acid, a bioactive GA widely utilized in agriculture to modulate plant growth. The fungal GA biosynthetic enzymes are encoded by a cluster of seven genes which include four P450 monooxygenase genes. Gene expression is repressed by ammonium and induced in the absence of nitrogen. Within the *G. fujikuroi* complex all the species contain GA biosynthetic genes in contrast to *Fusarium* species outside the complex that lack these genes. In this work *Fusarium graminearum*, a species distantly related to *F. fujikuroi* that does not belong to the complex, was complemented with the *F. fujikuroi* gene cluster in order to investigate if GAs are synthesized and if nitrogen repression is present. The activities of the GA oxidases were assayed with ^{14}C-labelled substrates in chemically defined liquid cultures containing ammonium nitrate or in the absence of nitrogen compounds. Several oxidation products were formed from ^{14}C-GA_{12} or from ent-^{14}C-kaurenoic acid as expected for reactions catalyzed by oxidases of the GA pathway. Metabolization of ^{14}C-GA_{12} was 7 times faster in cultures containing no nitrogen than in media containing 4.8 g/L ammonium nitrate. These results indicate that *F. fujikuroi* GA oxidases are expressed and are active in the genetic background of *F. graminearum* and are regulated by nitrogen in a similar way as *F. fujikuroi*. FONDECYT 1061127.

48.High concentrations of lipids alter mitochondrial dynamics and cause insulin signaling desensibilization in cultured cardiomyocytes. López-Crisosto, C.[1], Kuzmicic, J.[1], Morales, C.[1], Parra, V.[1], Castro, P.[2], Lavandero, S.[1]. Centro FONDAP Estudios Moleculares de la Célula, Facultad de Ciencias Químicas y Farmacéuticas y Facultad de Medicina, Universidad de Chile[1], División de Enfermedades Cardiovasculares, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile.[2].

Overweight and obesity are chronic conditions that have augmented considerably during the last decades. Both conditions are important risk factors for chronic pathologies such as diabetes and cardiovascular diseases. These last ones are the leading cause of death in Chile, underlying the importance of understanding the dyslipidemia-induced mechanisms involved in heart damage and insulin resistance. The heart is one of the most energy-consuming organs, requiring large amounts of ATP to account for contractility. Because of this, mitochondria are very important organelles in cardiomyocytes, forming dynamics networks that are remodeled by fission and fusion processes. The aim of this study was to investigate the effects of palmitate and ceramides on insulin signaling and mitochondrial dynamics in cultured rat cardiomyocytes. The results show that the treatment of cardiomyocytes with 500 μM palmitate for 3 h decreased phospho-Akt levels in response to insulin. Interestingly our confocal microscopy studies depict that palmitate also alters mitochondrial dynamics, causing a fragmented mitochondrial network in mitotracker green stained cells. Because palmitate could be metabolized to ceramides, we tested the effect of C_2-ceramides both in mitochondrial dynamics and metabolism. Our data show that C_2-ceramides (40 μM for 3 h) induce decreases in insulin-induced Akt phosphorylation, mitochondrial membrane potential, ATP levels and also stimulate mitochondrial network fragmentation. In conclusion, palmitate and ceramides alter mitochondrial dynamics, effect that could be linked to insulin signaling desensibilization. CLC, JK, CM and VP hold CONICYT scholarship. FONDAP 1501006, FONDECYT 1080436, 1090727.

49.Insulin promotes mitochondrial fusion and enhances mitochondrial metabolism in cultured skeletal muscle cells. **del Campo, A.**[1], Parra, V.[1], Gutiérrez, T.[1], Kuzmicic, J.[1], **Lavandero, S.**[1]. Centro FONDAP Estudios Moleculares de la célula. Facultad de Ciencias Químicas y Farmacéuticas y Facultad de medicina. Universidad de Chile.[1].

Insulin is the major regulator of energy metabolism in humans. This hormone stimulates glucose uptake and its utilization by the skeletal muscle. This organ plays an essential role in maintaining 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 linked to cell metabolism. In this work we investigate how insulin controls both mitochondrial dynamics and metabolism. L6-myoblasts were treated with 100 nM insulin for 30 min, 3 and 6 h and the stained with mitotracker green. To perform mitochondrial dynamics analysis, confocal microscopy images were taken and processed by ImageJ software. Oxygen consumption rate was measured with a Clarks? electrode and mitochondrial dynamics protein levels were determined by Western blot. Results show that insulin increased significantly improving oxygen consumption after 30 min and 3 h. Moreover this metabolic response was accompanied by increases in mitochondrial fusion and in the levels of the mitochondrial protein OPA1, a critical protein for the processes of mitochondrial fusion and cristae remodeling after 3 h. Our data also show that insulin stimulates mitochondrial calcium movements. Our future studies will investigate if this last event induced by insulin is associated with mitochondrial dynamics and metabolism in skeletal muscle cells. A del C and JK hold a CONICYT scholarship. FONDAP 1501006 FONDECYT 1080436 Mecesup UCH0802.

50.Participation of the *Escherichia coli* Pit phosphate transport system (PitAB) in tellurite uptake. **Elías, A.**[1], Abarca, M.J.[2], Vásquez, C.[1]. Universidad de Santiago de Chile[1], Universidad Nacional Andrés Bello[2].

Most of the known toxic effects of tellurite (TeO_3^{-2}) are intracellular, including depletion of the cellular pool of reduced thiols, inactivation of metabolic enzymes and the generation of reactive oxygen species. However, the available evidence about the routes underlying tellurite uptake are rather scarce. In a previous work, the participation of various transporters putatively associated with this phenomenon was analyzed. The results indicated that the main responsible for tellurite uptake was the Pit phosphate transport system. In this regard, tolerance to the toxic of cells lacking PitA or PitB transporters was assessed by analyzing tellurite intracellular effects such as thiol depletion and induction of superoxide dismutase activity. The ability of these transporters to mediate tellurite uptake by PitA/B-enriched right side out vesicles was assayed in vitro. Results strongly suggest that PitA is the main responsible for tellurite uptake in *E. coli*. Funded by Fondecyt #1090097 and Dicyt Usach.

51.Resveratrol affects glucose transport and accumulation in human HL60 and U937 leukemic cell lines. **Ojeda, M.L.**[1], Parada, D.[1], Castillo, B.[1], Vega, E.[1], Cea, A.E.[1], Pérez, A.[1], Reyes, A.M.[1], Salas Grandez, M.R.[1]. Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Campus Isla Teja, Valdivia, Chile.[1].

Interest in the biological actions of resveratrol (3,5,4-trihydroxystilbene; RSV) arises from its identification as a chemo preventive agent for skin cancer, its cardio protective effects, and also because it activates sirtuin deacetylases and extends the lifespans of lower organisms. We show that RSV decrease proliferation of HL60 cells and our interest is to further study their biological effect on leukemic cells. Human leukemic cells HL-60 and U937 are able to transport glucose through the glucose transporters GLUT1 and GLUT3. We performed a detailed kinetic analysis of the uptake of deoxyglucose (DOG) by HL-60 and U937 cells to differentiate transport from the intracellular phosphorylation/ accumulation. A linear kinetic component was observed when the time-course of the uptake of DOG was measured at short times (30 s). The amount of cell associated radioactivity increased rapidly in the first minute and decreased afterward showing a second kinetic component that was linear for at least further 60 min. The initial lineal phase represented DOG transport through GLUTs, whereas the second component corresponds to DOG intracellular accumulation. In zero-trans entry assays at short times (30 s) RSV blocks transport with IC50 values of 70 uM and 53 uM for HL60 and U937cells, respectively. At longer times (40 min) to evaluate accumulation, we obtain an IC50 of 38 uM and 53 uM for HL60 and U937 cells, respectively. Therefore, RSV blocks GLUT-mediated glucose uptake and independently affects cellular substrate accumulation. Fondecyt 11090404, FONDEF D07I1117, DID-UACH.

52.Effect of tellurite on the *Escherichia coli* NADH dehydrogenase I complex and NADH dehydrogenase II activities. **Díaz Vásquez, W.A.**[1], Abarca Lagunas, M.J.[1], Vásquez Guzmán, C.C.[1]. Facultad de Química y Biología, USACH[1].

Tellurite is toxic -even at very low concentrations- for most organisms, especially Gram-negative bacteria. In *Escherichia coli*, tellurite-induced damage is in part caused by reactive oxygen species (ROS). Some metabolic enzymes exhibit -as a branch activity- the ability to reduce tellurite to elemental tellurium, which is by far less toxic than tellurite. During toxicant reduction (generally NADH-dependent) superoxide is concomitantly generated. Interested in cellular targets of tellurite and given the NADH dependence of the respiratory membrane complexes, membrane-enriched fractions were assayed for tellurite reduction. Native PAGE revealed a new tellurite-reducing activity. A putative candidate for this reducing activity may lay in the *E. coli* electron transport chain (ETC), composed by two dehydrogenases and three cytochrome oxidase complexes. Membrane fractions from tellurite-exposed cells showed decreased oxygen consumption, NADH dehydrogenase activity and increased superoxide production. Finally, the relative transcript level of ETC genes was determined by qPCR. Results suggest that expression changes of respiration-regulating genes occur in *E. coli* during tellurite exposure. Funded by Fondecyt 1090097 and Dicyt USACH.

53.Participation of the acetate permease transporter (*actP*) in tellurite uptake by *Escherichia coli*. **Abarca Lagunas, M.J.**[1], Elías Bustos, A.[1], Vaquez Guzman, C.[1]. Facultad de Química y Biología, USACH[1].

Tellurite is highly toxic for most organisms. Once inside the cell, tellurite is initially reduced to elemental tellurium, which is much less harmful to bacteria. Associated with this reduction is the generation of reactive oxygen species (ROS), which display multiple intracellular targets. However, the mechanisms involved in tellurite entrance are still unknown.To date, several tellurite uptake mechanisms have been proposed in different bacterial models. While some of them suggest that the uptake is a pH-dependent process, other state that tellurite uptake occurs through phosphate or monocarboxylate transporters. In this work, the involvement of the ACTP acetate transporter, encoded by the *actP* gene, in tellurite entry to *E. coli* was assessed by analyzing tellurite uptake, growth inhibition zones and antioxidant enzyme activities. Funded by FONDECYT 1090097 and DiCYT USACH.

54.Effect of potassium tellurite on the *Escherichia coli* cytochrome oxidase complex activity. **Abarca Lagunas, M.J.**[1], Díaz Vásquez, W.A.[1], Vásquez Guzmán, C.C.[1]. Facultad de Química y Biología, USACH[1].

The tellurium oxyanion tellurite is toxic for most microorganisms, especially Gram negative bacteria. The induced damage if often associated with reactive oxygen species (ROS) generation.To date, very little is known about the effect of tellurite on the central oxidative metabolism, specifically on the electron transport chain (ETC). The bacterial ETC is composed by three oxidase (cytochrome *bo, bd-I, bd-II*) and two dehydrogenase complexes, which use NAD(P)H to reduce oxygen. While the electrochemical gradient is used for ATP synthesis by the ATPase complex, ROS are produced as unwanted by products during normal respiration.Some metabolic enzymes can reduce tellurite to elemental tellurium in vitro, with the concomitant generation of superoxide. In this context, the accumulation of black deposits in membranes from tellurite-exposed cells has been described. This process was associated with ETC terminal oxidases, making them potential tellurite targets.In this work, cytochrome oxidase activity was assayed in membrane fractions from tellurite-exposed cells. *E. coli* lacking genes encoding the catalytic subunit of each complex was also used as membrane source. Assays were carried out using synthetic electron donors and superoxide generation was assessed using the oxidation-sensitive WST-1 reagent. Finally, transcript levels of CTE genes were determined by qPCR. Results are compatible with changes of respiration-regulating genes in tellurite-exposed *E. coli*. Funded by Fondecyt 1090097 and Dicyt USACH.

55.Changes in protein expression levels of metabolism and drug resistance proteins after treatment with flumequine and florfenicol antibiotics in Chinook salmon embryo cells (CHSE-214) cultured in vitro. **Carreño, C.**[1], Barrientos, C.[1], Aguilar, M.[1], Castilla, S.[1], Manriquéz, R.[1], Villalba, M.[1], Cárcamo, J.G.[1], Yáñez, A.[1]. Universidad Austral de Chile[1].

The effect of two antibiotics commonly used in salmon farming, flumequine and florfenicol on the expression levels of metabolism and drug resistance proteins (MDRs) in CHSE-214 was assessed.We evaluated by RT-PCR and Western blot the effect on the expression levels of proteins involved in drug metabolism (CYP1A, CYP3A, and GST FMO) and proteins involved in xenobiotic transport output (Pgp and MRP1), post Flumequine application (10 mg / mL) or florfenicol (10 mg / mL) at different incubation times (24, 48 and 72 hours). Also, the effect of antibiotics on cell viability was quantified by MTT assays. Compared to control without treatment, we observed fluctuations in the levels of mRNA expression of all proteins analyzed after the cell treatment with flumequine and florfenicol. Under our conditions, antibiotics showed no effect on the viability of CHSE-214 cells.These results show that flumequine and florfenicol induce changes in the expression of metabolism and drug resistance proteins in CHSE-214 cells, which may be associated with the induction of drug resistance phenomena after drug therapy. This study also enables further about the mechanisms involved in the phenomenon of drug resistance and its impact at the aquaculture and public health.

56.Regulation of chaperone-mediated autophagy and its role on cardiomyocyte survival. **Toro Pavez, B.D.**[1], Lavandero Gonzalez, S.A.[1]. Centro FONDAP Estudios Moleculares de la Célula, Facultad Ciencias Químicas y Farmacéuticas/Facultad de Medicina, Universidad de Chile.[1].

Chaperone-mediated autophagy (CMA) is a degradative pathway activated by nutrient deprivation. Our aim was to study cardiomyocyte CMA regulation and its relationship with survival and autophagy. Primary cultured cardiomyocytes were subjected to serum/glucose deprivation for different times. Our data showed that nutrient deprivation increased Lamp 2A and Hsp 70 levels, assessed by Western blot and immunofluorescence. However, such increase in Lamp 2A was prevented by the AMPK inhibitor compound C (1nM) and cycloheximide (70 uM) but not by rapamycin (100 nM) or 3 methyladenine (3-MA, 10 mM). Nutrient deprivation increased both reactive oxygen species (ROS) and polyubiquitinated proteins and decreased GSH and NADH/NADPH levels after 18 and 24 h. The precursor of GSH N acetylcysteine (NAC, 5 mM) did not abolish Lamp2A level increase induced by nutrient deprivation. CMA dependent protein degradation (CDPD) was studied by pulse and chase experiments. CDPD increased significantly in 24 h nutrient starved cardiomyocytes. The decrease in Lamp 2A levels (60%) by a siRNA decreased cardiomyocyte death induced by nutrient deprivation and the viability decreased more with 3-MA. Under the same condition, autophagy was activated as shown by GFP-LC3 degradation and LC3B processing. Collectively these data show that nutritional stress triggers both autophagy and CMA and cell death decreased when CMA is inhibited, effect that it is prevented by 3-MA. We conclude that autophagy activation is a protective event and there is a relationship between autophagy and CMA. FONDAP 15010006. BT es becaria CONICYT.

57.Malin and laforin form an active complex to regulate the muscle glycogen synthase isoform in Sertoli cells. Vander Stelt, K.[1], Villarroel-Espíndola, F.[1], Maldonado, R.[1], Torres, C.[1], Angulo, C.[1], Castro, M.A.[1], Slebe, J.C.[1], García-Rocha, M.[2], Guinovart, J.J.[2], Concha, I.I.[1]. Instituto de Bioquímica y Microbiología, Universidad Austral de Chile[1], Institut de Recerca Biomédica de Barcelona, España[2].

The blood-testis barrier has been described as a structural and metabolic support for germ cells. The barrier is formed by Sertoli cells, which should exhibit a complex regulation of glycogen metabolism. Glycogen is the main energy reserve for the cell and its synthesis and degradation are highly regulated processes. Various strategies have been reported for the regulation of both glycogenesis and glycogenolysis: changes in subcellular localization, allosteric regulation and covalent modifications (phosphorylation/ dephosphorylation) of the enzymes involved in each process. Our laboratory has demonstrated that the muscle isoform of glycogen synthase (MGS) is expressed in Sertoli cells, as well as the regulatory proteins Malin and Laforin, described in Lafora disease, which modulate MGS activity in neurons through polyubiquitination and proteosomal degradation. The aim of this work was to evaluate the interactions of these proteins and verify the capability of the complex Malin-Laforin to regulate the MGS in Sertoli cell line 42GPA9. Colocalization of Malin and Laforin, and these with MGS were clearly observed by confocal microscopy. Additionally, treatment of Sertoli cells with MG-132 and cycloheximide showed an accumulation of polyubiquitinated MGS. These results indicate that in Sertoli cells, Malin and Laforin form an active complex capable of regulating MGS availability through polyubiquitination and proteosomal degradation, suggesting that the mechanism described for central nervous system (Lafora disease) could be a strategy used in other tissues to regulate glycogen homeostasis.(CONICYT FV y AT-24100011; MECESUP AUS-0704; FONDECYT-1110508, 1090740 y 1110571; DID-UACh).

58.Absence of the gluconeogenic enzyme fructose-16-biphosphatase in Sertoli cells and subcellular muscle glycogen synthase localization under different metabolic conditions. Torres, C.[1], Villarroel-Espíndola, F.[1], Maldonado, R.[1], Mancilla, H.[1], Barraza, R.[1], Siebe, J.C.[1], Concha, I.I.[1]. Instituto de Bioquímica y Microbiología, Universidad Austral de Chile[1].

Sertoli cells are very important for spermatogenesis and posses large amounts of glycogen, the main glucose reservoir in higher organisms. This polymer can be synthesized by two pathways: the direct pathway, which uses glucose absorbed by the cell, and the indirect pathway, which uses gluconeogenic precursors. Both pathways are very important to maintain an adequate level of glycogen. Glycogen Synthase (GS) (both pathways) and Fructose-1,6-Biphosphatase (FBPase) (indirect pathway) change their subcellular localization depending on the metabolic condition of the cell. To determine subcellular localization changes of these enzymes and analyze its correlation with Sertoli cell glycogen synthesis, RT-PCR, Western Blot and immunohistochemical assays were made, using 42GPA9 Sertoli cells. The results showed that FBPase is not expressed in this cell type, suggesting the absence of a gluconeogenic pathway, which may indicate a high lactate biodisponibility to be used by germ cells. On the other hand, Sertoli cells cultured under different conditions (with or without glucose, in high glucose and in IGF-1) did not show changes of GS subcellular localization. This enzyme only changes its localization in the presence of LiCl, condition in which it is mainly found in cytosolic granules and in the nuclei, co-localizing with SC-35. This study indicates for first time that glucose and glycogen metabolism in Sertoli cells may be different to other glycogen synthesizing cell types, like hepatocytes and muscular cells. (FONDECYT 1090740 y 1110508, DID-UACh).

59.Role of mitochondrial dynamics on the pathophysiology of cardiomyocyte hypertrophy. Pennanen, C.[1,2], Rivera, P.[2], Morales, P.[1,2], Parra, V.[1,2], Chiong, M.[1,2], Lavandero, S.[1,2,3]. Centro FONDAP Estudios Moleculares de la Célula[1], Facultad Ciencias Químicas y Farmacéuticas, Universidad de Chile[2], Facultad de Medicina, Universidad de Chile[3].

Work overload induces cardiomyocyte hypertrophy initially as an adaptative physiological process, but later on as a pathological response producing ventricular dysfunction and chronic heart failure. Both physiological and pathological responses are associated to an increased and decreased mitochondrial activity, respectively. Because mitochondria exist in a dynamical equilibrium between fusion and fission, we investigate here the involvement of mitochondrial dynamics and bioenergetics in both physiological and pathological cardiomyocyte hypertrophy. To this end, primary cardiomyocyte cultures were exposed to norepinephrine (NE, 10 μM, 0-48 h) and insulin-like growth factor-I (IGF-1, 10 nM, 0-48 h) as pathological and physiological stimuli, respectively. Mitochondrial morphology was determined using mitotracker green staining and confocal microscopy. Our data show that NE, but not IGF-1, induced mitochondrial network fragmentation and decreased mitochondrial function, evaluated as O2 consumption rate (OCR), mitochondrial potential ($\Delta\Psi m$) and ATP levels. Mitochondrial dynamics was manipulated using adenoviral overexpression of antisense Mfn2 fusion protein (asMfn2) and dominant negative Drp1 fission protein (DrpK38A). AsMfn2 induced cardiomyocyte hypertrophy determined by increases in cell size and β-MHC protein levels while DrpK38A decreased both IGF-1 and NE-induced hypertrophy. These results suggest that mitochondrial dynamics plays an important role in the generation of cardiac hypertrophy and mitochondrial fission occurs only in pathological growth. MECESUP UCH0802, FONDAP15010006. CP, VP and PM hold CONICYT fellowships.

60.Effect of oxidative stress on the vernalization response using several Arabidopsis ecotypes that express the *FRI/FLC* module. Moraga, F.[1], León, G.[1]. Laboratory of Plant Reproduction & Development, Center of Plant Biotechnology, Andrés Bello University [1].

Flowering in plants is under a tight genetic and environmental control, and the most important pathways regulating this process are the photoperiod and the vernalization. Both converge in the master regulatory gene *FT (FLOWERING LOCUS T)*, 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 and Ler, don't have a functional *FRI/FLC* module. In this work we've 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 for sub-optimal cold treatments. proFLC:GUS transgenic plants show less GUS histochemical activity when growth on salicylic acid (SA), which indicate that this response is controlled at the transcriptional level. Therefore, is expected that levels of *FLC* transcripts decrease 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 UNAB DI-23/10-R.

61.Oxidative stress affects the functions of Glutamyl-tRNA synthetase and Glutamyl-tRNA Reductase and favors the channeling of glutamyl-tRNAGlu to the biosynthesis of proteins instead of tetrapyrroles. Farah, C.[1], Katz, A.[2], 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].

Acidithiobacillus ferrooxidans, an acidophilic and autotrophic gamma proteobacteria, uses Glu-tRNAGlu, formed by the glutamyl-tRNA synthetase (GluRS) as a common substrate for the biosynthesis of tetrapyrroles (heme) and proteins. In the biosynthesis of heme, the glutamate moiety of Glu-tRNAGlu is reduced by the glutamyl-tRNA reductase (GluTR) to glutamate semialdehyde (GSA). Then GSA is transformed to aminolevulinic acid (ALA), the universal precursor of the biosynthesis of heme, by glutamate semialdehyde amidotransferase (GSAM). When Glu-tRNAGlu goes to the biosynthesis of proteins, it binds to the elongation factor Tu (EFTu) to be delivered to the ribosome. In *A. ferrooxidans* the biosynthesis of heme might be negatively affected by oxidative stress since hydrogen peroxide inactivates GluRS either *in vitro* or *in vivo* and decreases the activity of GluTR *in vitro*. Competition assays between GluTR and EFTu suggested that Glu-tRNAGlu might be functionally channeled to the biosynthesis of proteins under oxidative stress. Supported by Fondecyt 1070437 and 1110203 (OO) and Becas Chile (CF).

62.Measurement of ROS in *Salmo salar* inoculated with prototype vaccine against *Piscirickettsia salmonis*. Espinoza, C.[1,2], Troncoso, J.M.[2], Valenzuela, K.[3], Pontigo , J.P.[3], Yáñez, A.J.[3], Olavarría, V.H.[3]. Departamento de Ciencias Pecuarias, Universidad de Concepción, Concepción, Chile.[1], EWOS Innovation S.A.[2], Instituto de Bioquímica y Microbiología, Universidad Austral de Chile, Valdivia, Chile. [3].

The greatest economic losses in the Chilean salmon aquaculture have been produced by salmonid rickettsial septicaemia (SRS). This disease is caused by a facultative intracellular pathogen, *Piscirickettsia salmonis*. The aim of research was to evaluate the effect of a prototype vaccine in the salmon innate immune system. Five groups of Atlantic salmon (*Salmo salar*) were immunized by intraperitoneal injections with different vaccine prototypes using three different proteins extract, which were used in two doses. Three salmon for each group were sacrificed at 2, 3, 7 and 9 days post-inoculation and there kidneys was collected to isolate primary culture total kidney cells. These cells were used to measured the generation of ROS (Reactive Oxygen Species) through luminol-dependent chemiluminescence methods. The results showed that production of reactive species partially increase on different days and doses, suggesting an activation of the immune innate response against the different commercial and vaccine prototypes tested in this trial. These results suggest that the stimulation of ROS is produced with lower doses of antigen and also with the adjuvant, indicating that this metabolite is not a good vaccine marker. Supported by: INNOVA-CORFO 07CN13PPT-256 and EWOS Innovation.

63.Role of glutaredoxin GRXS13 in response to photooxidative stress in Arabidopsis. Olate, E.[1], Laporte, D.[1], Salazar, M.[1], Holuigue, L.[1]. Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile[1].

The enzymatic redox systems are essential for maintenance of cellular redox homeostasis. Glutaredoxins (GRX) are small oxidoreductases involved in cellular redox processes. In spite of the high number of GRX genes in plant genomes, the biological functions and physiological roles of most of them remain unknown. Here we report the functional characterization of the Arabidopsis GRXS13 gene. We showed that *GRXS13.2* gene variant is activated in response to biotic an abiotic conditions, such as avirulent strains of *Pseudomonas syringae* pv. tomato (Pst), low temperature and photooxidative stress such as high light (HL) and methyl viologen (MeV) treatments in Arabidopsis plants.To determine the role of GRXS13.2 in the cellular response to oxidative stress produced by HL and MeV treatments, we have analyzed redox status, tolerance, and oxidative damage in different transgenic lines that silence and over-express the *GRXS13* gene. Silenced lines showed more accumulation of superoxide ion levels under basal and stressed conditions and reduced tolerance to MeV and HL treatments, in comparison to wild type plants. On the other hand, transgenic plants that over-express this gene showed a significant reduction in the HL- and MeV-induced damage. These results indicate that GRXS13.2 shows a particular low functional redundancy in the GRXs family, playing an important role in protection and tolerance to photooxidative stress conditions in Arabidopsis. FONDECYT-CONICYT (1100656) and Millennium Nucleus for Plant Functional Genomics (P10-062-F).

64.Effect of tellurite-mediated oxidative stress on the *Escherichia coli* glycolytic pathway. Valdivia, M.[1], Díaz, W.[2], Sabotier, M.[1], Perez, J.M.[2], Vasquez, C.[2]. Universidad Andres Bello[1], Universidad de Santiago de Chile[2].

Glycolysis is a central pathway of the cell metabolism that plays two essential roles. First, glucose-6-phosphate is oxidized to generate ATP, NADH and pyruvate and second, it functions as an amphibolic route. Even when in *E. coli* most glycolytic reactions are reversible, those catalyzed by phosphofructokinase or pyruvate kinase are not. Thus, any damage generated at this level generates a negative effect on this metabolic pathway. In this context, different metals affect specifically glycolysis and in this study we assessed the effects of the tellurium on the expression and activity of key enzymes of the *Escherichia coli* glycolytic pathway. *E. coli* exposure to tellurite results in increased glucose consumption, which was paralleled by increased expression of the glucose transporter-encoding gene *ptsG*. Augmented phosphogluco isomerase activity and *pgi* transcription occurred, while the activity of the enzymatic regulators phosphofructokinase and pyruvate kinase was significantly decreased. In spite of these observations, increased intracellular pyruvate, phosphoenolpyruvate and phosphorylated sugars concentrations were observed. *E. coli* cells lacking key glycolytic enzymes were considerably more sensitive to tellurite than the parental, wild type strain. Taken together, these results suggest that to increase the availability of key metabolites (pyruvate, phosphoenolpyruvate, NADPH) required to respond to tellurite mediated-stress, *E. coli* shifts the metabolic carbon flux towards the pentose phosphate pathway augmenting the activity of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, thus facilitating the functioning of the Entner-Doudoroff pathway and/or the glycolytic productive phase.Financial support from Fondecyt 1090097 and Dicyt-USACH.

65.Glucagon like peptide-1 (GLP-1) promotes mitochondrial fusion in vascular smooth muscle cells. Torres, G.[1], Morales, P.E.[1], Michea, L.[2], Lavandero, S.[1,2], Chiong, M.[1]. Centro FONDAP Estudios Moleculares de la Célula, Facultad de Ciencias Químicas y Farmacéuticas/Facultad de Medicina[1], ICBM, Facultad de Medicina, Universidad de Chile[2].

Mitochondrial morphology changes continuously due to fission and fusion events. Mitofusins and Opa-1 control fusion and glucose metabolism whereas fission is regulated by Drp-1 and Fis 1 and associated to cell death. PKA inactivates Drp-1 by phosphorylation on Ser637, promoting mitochondrial fusion. The incretin GLP-1, used in diabetes treatment, promotes glucose metabolism in several cell types. However, its effect on VSMC remains unknown. GLP-1 receptor activation is coupled to adenyl cyclase/cAMP/PKA signaling pathway. Our aim was to determine whether GLP-1 controls mitochondrial dynamics on the rat aortic VSMC cell line A7r5. Cells were exposed to GLP-1 for 0 24 h 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. Phospho-CREB, CREB, phospho-Ser637-Drp-1 and Drp-1 levels were determined by Western blot. Our data showed that GLP-1 promotes mitochondrial fusion because increased average volume and decreased particle number per cell. Also, GLP-1 increased phospho-CREB levels (a known PKA target). Phospho-Ser637-Drp-1 levels correlated with mitochondrial fusion. In summary, our results suggest that GLP-1 promotes mitochondrial fusion in VSMC, possibly by inhibiting Drp-1 function. FONDECYT 1110180 (MC); FONDAP 1501006 (SL, MC, LM), Becaria Doctorado CONICYT (GT), Becario Magister CONICYT (PM).

66.Effect of temperature on C-phycocyanin, a molecular explanation. Morales, M.[1], Martínez-Oyanedel, J.[1], Bunster, M.[1]. Laboratory of Molecular Biophysics, Dept.Biochemistry and Biology, Faculty of Biological Sciences. Universidad de Concepción, Chile.[1]

C-Phycocyanin (C-PC) is a phycobiliprotein present in *Gracilaria chilensis*. Its oligomeric structure $(\alpha\beta)_6$ contains 18 phycocyanobilins, associates to Cys 84 in α subunits and to Cys 82 and Cys 153 in β subunits. The spectroscopic properties of C-PC (λ_{max}^{abs} 621 nm and λ_{max}^{em} 634 nm), besides their natural function of light harvesting and energy transfer in phycobilisomes, are used for biotechnological applications, which depends on the stability of the system. These spectroscopic proprieties are affected by variation of temperature, been this once of limitation in his clinical and biologic application like fluorescent marker.C-PC, purified from Gracilaria chilensis was used to study the effect of temperature (from 20°C to 60°C) on the spectroscopic properties of phycocyanin. The absorption and emission spectra was recorded using a temperature gradient, with incubation periods of 10 min, showing not only the transition but differential decay in the two absorption maxima of phycocyanin (550 nm and 621 nm) and the lost of fluorescence .The transition was also simulated in silico, using the tri dimensional structure of this protein available in Protein Data Bank ID Code 2bv8 and GROMACS software, using a protocol that mimic the experimental protocol for a total of 1 ns at each temperature. Using the results from both protocols, a molecular explanation for the changes is proposed. **Funding: FONDECYT 108.0267** .

67.Probabilistic method to analyze stability of membrane proteins in the gas phase. Montecinos, A.[1], Barrera, M.[1], Montenegro, F.[1], Torres, S.[2], Barrera, N.P.[1]. Department of Physiology, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile[1], Department of Statistics, Universidad de Valparaíso[2].

We have discovered that intact membrane proteins can be detected in vacuum via Mass Spectrometry. This process involves first the transfer of membrane protein-micelle complexes (MPMCs) from solution into gas phase and second the release of the protein after MPMCs are collided with gas molecules. However the molecular mechanism for the micelle destruction is poorly understood. We propose that a new probabilistic model based on MPMC geometrical properties can be applied to the transfer of internal energy occurring after collision. Using the solvent accessible surface area (SASA) calculated for each residue and detergent molecule in the complex, we can provide a higher probability of collision at larger SASA values. Considering the center of mass for each component (residue and detergent) and the distance between them we can estimate the sequence of collisions events and the internal energy transfer between components. As proof of principles we have used the tetrameric BtuCD transporter complexed to DDM detergent as our working model. In addition we have corroborated collision event positions via nano-mechanical simulations of the MPMC collided to gas molecules. We expect this approach can be applied to simulations of the stability of protein complexes in a complementary way to molecular dynamics (MD) studies. Fondecyt Grant 1100515 and Wellcome Trust 088150/Z/09/Z.

68.Transmembrane Domains M2 y M4 Of TASK-2 are implicated in the binding site of Quinidine, a antiarrhythmic agent. Marchant, C.[1], Martinez, G.[1], González-Nilo, D.[1,2], **González, W.**[1]. Universidad de Talca.[1], the Centro Interdisciplinario de Neurociencia de Valparaíso is a Millennium Science Institute.[2].

The K_2P potassium channels are important regulators of many physiological processes in cell of the cardiovascular system, the central nervous system and adrenal gland. These channels are composed of dimer, where each subunit present two domains of pore and four domains transmembrane (M1-M4). In mammalias have identified 15 gene (KCNK) encoding for K_2P channels. These proteins are associated with a variety of human pathologies therefore have emerged as candidates molecular for the action of agent drug. However, these channels have a poor selectived pharmacological so that more of K_2P channel is inhibited or activited for the same drugs, although to different grade. Little is know about the structure of these channels and the binding site agents pharmacological. Previuos studies have demostrated that the C-terminal domain of the channels K_2P play a role important in the modulation of local anaesthetic. But this domain is not sufficient by itself. In this work we will studied specifically the K_2P potassium channels TASK-2 and TASK-3 and inhibition by quinidine. This will involved chimeric protein of TASK-2, containing the segments transmembrane M2 and M4 of TASK-3 to determine the domains that are involved in binding site of quinidine. Through electrophysiological analysis the chimeras, will show that the segments M2 and M4 are important elements for binding of pharmacological agent. Fondecyt 11100373, The Centro Interdisciplinario de Neurociencia de Valparaíso is a Millennium Science Institute.

69.Lipid binding effect on membrane proteins inserted in bilayers and bound to detergent micelles. Alveal, N.[1], Brugues, G.[1], Barrera, N.P.[1]. Department of Physiology, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile[1].
Membrane transporters play important roles for cell-to-cell communication and antibiotic resistance in bacteria. The ABC transporter Sav1866 in Staphylococcus aureus has been crystallized as a dimer and has been proved to act as multidrug transporter. We have confirmed its dimeric composition via Mass Spectrometry and stoichiometric cardiolipin (CL) binding has been detected on the intact protein in the gas phase. Surprisingly non binding is observed in the monomeric specie which suggests that binding sites could be located in subunit interfaces. However it remains to be analyzed the effect of lipid binding on the stability of the transporter. We have carried out molecular dynamics (MD) simulations of the transporter Sav1866 inserted in a lipid bilayer and complexed to a $C_{12}E_8$ detergent micelle. In addition docking studies have been performed using two CL molecules bound to the subunit interfaces. We have found that lipid binding affects protein stability in both systems in a characteristic way. Fondecyt Grant 1100515 and Wellcome Trust 088150/Z/09/Z.

70.Covalent modifications in components of the vitamin D receptor-associated complex. 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 super family of nuclear receptors and functions in the mammalian nucleus as a transcription factor, modulating the expression of target genes in response to the ligand vitamin D. Ligand binding at the C-terminus of VDR promotes conformational changes that allow interaction with transcriptional coactivators like the members of the p160/SRC family and components of the DRIP/TRAP/Mediator complex (DRIP205). It has been described that the interaction of the estrogen receptor with coactivators is controlled by specific post-translational modifications occurring at both the receptor molecule and coactivators. Therefore, we have explored this regulatory mechanism by addressing whether phosphorylation of VDR by CKII kinase and sumoylation of p160/SRC co-activators, contribute to regulate the formation of transcriptional regulatory complexes in osteoblastic cells. By combining site-directed mutagenesis, transient over-expression and co-immunoprecipitation, we conclude that phosphorylation of serine residue 208 of VDR can be an important component during the regulation of the VDR-coactivator interactions. Together, our results indicate during exposure of osteoblastic cells to vitamin D, specific signaling pathways are activated leading to post-translational modifications that facilitate the formation of regulatory complexes containing VDR that modulate transcription of target genes. CONICYT Fellow (P.M), FONDAP 15090007, FONDECYT 1095075(M.M).

71.Release of membrane proteins from detergent micelles in solution and gas phase. Montenegro, F.[1], Barrera, N.P.[1]. Department of Physiology, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile[1].
Structural biology on membrane proteins has been an evolving field over the years. Purified membrane proteins in solution are usually complexed to detergent micelles to maintain their solubility. Recently we have discovered a new method based on Mass Spectrometry to analyze the structure of intact membrane proteins in the gas phase. Membrane protein-micelle complexes (MPMCs) are collided to gas molecules at high kinetic velocity to induce selective removal of the micelle and to release the intact naked protein. This process is analogous to increasing the system temperature in molecular dynamics (MD) simulations. However the precise molecular mechanism of this process is unknown. We present here MDs of two MPMCs, BtuCD-DDM and KirBac3.1-TriDM, which are an ABC transporter and K^+ channel respectively. BtuCD-DDM complex in solution has a lower protein-micelle interaction energy compared to the KirBac3.1-TriDM complex. After increasing the temperature, the former complex releases the naked protein at a lower temperature which is in accordance to the increased instability of the BtuCD transporter compared to the KirBac3.1 channel in the gas phase. Interestingly MDs in the gas phase of the KirBac3.1-TriDM complex have shown that the complex is more stable in the gas phase than in solution. Therefore a considerable increase of system internal energy is required to release the naked protein in vacuum. We anticipate that these results will guide future experiments to make suitable MPMCs for structural studies. Fondecyt Grant 1100515 and Wellcome Trust 088150/Z/09/Z.

72.Crystallization and X - ray analysis of the ADP-dependent glucokinase from *Thermococcus litoralis* in the close conformation. Herrera-Morandé, A.[1,2], Rivas-Pardo, J.A.[2], Fernández, F.J.[1], Guixé, V.[2], Vega , M.C.[1]. Centro de investigaciones Biológicas, CSIC, Madrid, [1], Departamento de Biología, Facultad de Ciencias, Universidad de Chile[2].
Glucokinase from the hyperthermophile *Thermococcus litoralis* (TlGK) catalyzes the phosphorylation of glucose to yield glucose-6-phosphate. As a special feature, unlike most glucokinases, TlGK uses ADP instead of ATP as the phosphoryl donor. Also, as this enzyme is from a hyperthermophile microorganism, its structure is adapted to perform its activity at temperatures around 90° C. The resolution of the three-dimensional structure of TlGK in the presence of ADP allowed its classification as a member of the Ribokinase superfamily.Previous results of enzyme kinetics and SAXS suggest that the protein suffers a conformational change from an "open conformation" in the absence of ligands, to a "semi-closed" one in the presence of ADP. Finally, the "close conformation" is reached when both glucose and ADP are bound to the active site. In order to demonstrate that binding of both ligands causes conformational changes, the crystal structure of TlGK in the presence of glucose and the non-hydrolyzable ADP analogous (ADPβS) was obtained to simulate the formation of the ternary complex E-A-B. The 2.45 Å resolution structure generated allowed us to acquire information about the amino acids involved in the stabilization of each ligand, ADP and glucose, in addition to observe the changes in the packaging of the ternary complex. (BFU2010-22260-C02-02, PET2008_0101 projects, M. Cristina Vega; Ministerio de Ciencia e Innovación, España, and Fondecyt 1110137. We acknowledge the ESRF, Grenoble, France, for provision of the synchrotron radiation facilities).

73.Resurrecting an ancestral enzyme from the archaeal ADP-dependent sugar kinase family. Castro, V.H.[1], Merino, F.[1], Guixé, V.[1]. Laboratorio de Bioquímica y Biología Molecular. Departamento de Biología. Universidad de Chile.[1].

Promiscuous enzyme activities have been proposed as starting points for the generation of new specificities through evolution, so it is interesting to study the proteins that gave rise to the current enzyme specificities. Experimental paleogenetics and paleobiochemistry provide an opportunity to reconstruct the amino acid sequence of ancestral proteins by virtue of a comparison between the sequences of related proteins found in contemporary organisms to give an appropriate molecular evolution model. The ADP-dependent sugar kinases family has two specificities, phosphofructokinase and glucokinase. In this study we reconstructed the phylogenetic tree for the archaeal branch of this family using the Bayesian method. Also, we inferred, synthesized and expressed the gen of the last common ancestor for both specificities. As models for the current enzyme we have used the glucokinase from Thermococcus litoralis and the phosphofructokinase from Pyrococcus horikoshii. The 3300-3500 million years old ancestor showed K_m values similar to the modern enzymes, but with significantly lower k_{cat}. We built a homology model with which ligand-docking was performed with glucose and fructose-6-P. Then, molecular dynamics were performed to identify important residues for the binding and specificity of both substrates. These results are in good agreement with the hypothesis of ancestral "generalists" enzymes that are then specialized for their respective substrates. Fondecyt 1110137.

74.Biochemical and molecular analysis of tomato plants transformed with sorbitol dehydrogenase. Díaz, F.[1], Aguayo, F.[1], Araya, J.[1], Zamudio, S.[1], Handford, M.[1]. Laboratorio de Biología Molecular Vegetal, Departamento de Biología, Facultad de Ciencias, Universidad de Chile1. [1].

Sorbitol dehydrogenase (SDH) is the main enzyme involved in the metabolism of sorbitol in Rosaceae fruit species. This enzyme catalyses the reversible oxidation of sorbitol, a sugar alcohol which is the principal photosynthate translocated through the phloem and which has low sweetening power, to fructose, a sugar with enhanced sweetness. SDH thus plays an important role in naturally increasing fruit sweetness during development. This makes SDH an excellent candidate for modulating the composition of sugars in the fruit, given that the specific over-expression in this organ would lead to the oxidation of the sorbitol which is not metabolised during the ripening process.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, promoters which we have shown to be functional.Subsequently, tomato plants, as a model system, were stably transformed using *Agrobacterium tumefaciens* harbouring these vectors and potential transformants were selected on media containing antibiotic. Molecular analysis determined that these transformants effectively possess the transgene inserted in the tomato genome and biochemical assays will be undertaken once these plants bear fruit. In the meantime, we have shown using transient fruit transformation, that there is greater SDH activity in fruits which have been Agrobacterium-infiltrated with the constructs generated.Funding: Innova-Corfo 07CN13PBD-19.

75.Role of the conserved GAGD and NXXE motifs and phosphate 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) (E.C. 2.7.1.5) belongs to the ribokinase superfamily and catalyzes the phosphorylation of D-ribose using MgATP as cosubstrate, producing D-ribose-5-P and ADP. Even though ribose acts in important metabolic steps, kinetic studies of human RK are poor and preliminary. In this superfamily there are two conserved motifs, ^{199}NXXE^{202} and ^{266}GAGD^{269}, both localized in the active site. GAGD contains an aspartic residue (D269) that should act as a catalytic base, and NXXE is related with the proper use of Mg^{+2} and PO_4^{-3} ions. We have studied the role of residue D269 and the effect of PO_4^{-3} on the enzymatic activity. The D269N mutation leads to a reduction of the k_{cat} value, which is 84 times lower than that of the wild type enzyme. The K_m value for D-ribose decreases 10 fold due to the mutation, while the one for MgATP increases 10 fold. In contrast to the inhibition observed by both substrates in the wild type RK, the mutant shows no inhibition. Although PO_4^{-3} causes a 3.6 fold activation in the wild type RK, the activity of the D269N mutant was not altered in the presence of this ion. These results suggest that the D269 residue acts like a catalytic base in the human RK. Kinetic experiments with the mutant enzyme E202L show a greatly decreased activity, supporting the view that E202 plays an important role in the activity of RK. (Fondecyt 1110137).

76.A hybrid TIRF-magnetic tweezers instrument for studying sub-nanometer effects of force on proteins and DNA. Wilson, C.A.[1], Leachman, S.[2], Marqusee, S.[1], Bustamante, C.[1,3]. QB3 Institute, University of California at Berkeley[1], Chemical Biology Graduate Program, Department of Chemistry, University of California at Berkeley[2], Howard Hughes Medical Institute[3].

Proteins exert and withstand mechanical force in many fundamental biological processes. Optical tweezers have become a useful research tool for applying forces to single proteins and measuring the resulting changes in extension, but many interesting processes produce changes smaller (<1 nm) than their resolution limit. Our experimental setup skirts this limitation by measuring distance changes using single-molecule Förster resonance energy transfer (smFRET) produced from a total internal reflection fluorescence (TIRF) microscope incorporating magnetic tweezers. Individual protein molecules or DNA hairpins are conjugated to FRET-paired fluorescent dyes and functionalized DNA handles using maleimide and click chemistry. These handles tether each molecule between a glass coverslip within the TIRF microscope and a paramagnetic bead. An external magnet applies a uniform field that exerts a force on each protein or DNA hairpin tethered to the surface. Because the FRET from each molecule in the microscope's field of view can be measured simultaneously, the extension between dyes of many individual molecules as a function of force can be monitored in parallel. Here we present preliminary data of the DNA hairpin system used like a proof of principle for this machine.

77.FragProt, a database of short protein fragments clustered by structural similarity. 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, Alameda 340, Santiago, Chile.[2].
Protein fragments play an essential role in many bioinformatics areas, like homology protein modeling, secondary and tertiary structure prediction and the study of sequence - structure relationships. In this study, we developed a repository containing thousands of protein fragments clustered by structural similarity. The database also includes information like accessible surface area per residue and two hydrophobicity coefficients associated to each aminoacid. Also, the groups of fragments can be analyzed using a weblogo to obtain different information related to the sequence diversity within the groups. The method used to cluster the fragments is called de novo clustering, which possess a high scalability rate, allowing to include a vast quantity of fragments with a low computational demand. The major aim of this database is to help scientists to identify different fragments with the same sequence and, on the other hand, different sequences related to a specific fragment conformation. Potential applications of this database are selection of specific fragments to be used as blockers in drug design or modification of specific residues on a fragment to obtain a better stability in an aqueous medium.
ACKNOWLEDGEMENTS: This research was funded by grants from FONDECYT (1110400) and ICM (P09-016-F).

78.Inhibition of monoamine oxidases by coumarin derivatives: synthesis, biological activity and computational study. Moya-Alvarado, G.[1], García-Beltrán, O.[2], Morales, N.[1], Dahech-Levenberg, P.[1], Reyes-Parada, M.[3,4], Nuñez, M.T.[4,5], Cassels, B.K.[6], Fierro, A.[1,4]. Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile. [1], Facultad de Ciencias, Universidad de Chile, Santiago, Chile.[2], Facultad de Ciencias Médicas, Universidad de Santiago de Chile, Santiago, Chile.[3], Instituto Milenio de Dinámica Celular y Biotecnología, Santiago, Chile.[4], Facultad de Ciencias, Universidad de Chile, Santiago, Chile[5], Facultad de Ciencias, Universidad de Chile, Santiago, Chile[6]. Sponsored by A. Moene.
Coumarins, also known as benzopyrones, are present in plants, microorganisms and animal sources. The structural diversity found in these derivatives has led to coumarins and their analogues to be a class of compounds with high biological interest. In recent years coumarin derivatives have been reported to have inhibitory activity and selectivity towards monoamine oxidase (MAO) [1]. MAO is a FAD-containing enzyme tightly bound to the mitochondrial outer membranes, which catalyzes the oxidative deamination of monoamines from endogenous and exogenous sources, affecting the concentration of monoaminergic neurotransmitters. MAO exists in two isoforms called MAO-A and MAO-B, which have different inhibitor selectivity and substrate specificity [2]. In this work a new series of coumarines were synthesized, evaluated as MAO inhibitors and docked into the enzyme active site. Some derivatives were selective MAO-B inhibitors. The main molecular interactions found at the active site were those established with aromatic residues. These experiments give us an approach to understand the biological activity of coumarins against both isoforms of MAO and guide future research towards the development of new potent inhibitors. References:[1] Pisani L, Muncipinto G, Miscioscia TF, Nicolotti O, Leonetti F, Catto M, Caccia C, Salvati P, Soto-Otero R, Mendez-Alvarez E, Passeleu C, Carotti A., J Med Chem. 2009 52(21):6685-706.[2] Edmondson DE, Binda C, Wang J, Upadhyay AK, Mattevi A. Biochemistry. 2009, 48(20):4220-30. AcknowledgmentsFinancial support from Fondecyt 11085002, 1090037, PDA-23 and ICM-FIC P05-001-F.

79.An evidence of odorant binding protein (OBP) in *Hylamorpha elegans* (Coleoptera: Scarabeidae) antennae. Mutis, A.[1], Palma, R.[2], Alvear, M.[1], Quiroz, A.[1]. Departamento de Ciencias Químicas y Recursos Naturales, Universidad de La Frontera, Temuco[1], Programa de Doctorado en Ciencias de Recursos Naturales, Universidad de La Frontera, Temuco[2].
Scarab beetles are a widespread taxon distributed in all type of environments. In Chile, the larvae of *Hylamorpha elegans* Burmeister cause severe agricultural damage by feeding on roots of pastures and cereal crops. Biological and chemical control using agrochemicals has been unsuccessful for *H. elegans*. Despite the use of semiochemicals, as well the mechanism of perception of these compounds have been proposed for other scarab beetles belonging to the Rutelinae and Melolonthinae subfamilies , up to date, the single one report indicate that unmated females release 1,4-hydroquinone and 1,4-benzoquinone that attracted conspecific males of *H. elegans*. In this direction, the research has been focused on the molecular basis of perception and inactivation of pheromone, considering it is essential to figure out how H. elegans find its host and mate. The use of this knowledge would make possible to develop new control strategies.The analysis of protein extracts from antennae compared to control tissues of males and females of *H. elegans* by native polyacrylamide gel electrophoresis (PAGE) showed three antenna-specific proteins in males and two antenna-specific proteins in females, besides their mobility and migration pattern was similar to those of OBPs identified to date. Considering these results digested spot from males antenna-specific protein isolated from SDS-PAGE, were analyzed by HPLC-MS/MS, showed similar profiles to others odorant binding proteins previously described. Further studies to confirm the identity of this protein are being developed.Acknowledgments: Project DIUFRO DI10-018 and Postdoctoral Project CONICYT 3110062.

80.Structural and Biophysical Characterization of Fur (Ferric Uptake Regulator) from an Extreme Acidophile Thriving in an Iron Rich Environment. Arenas, M.A.[1,2,3], Marquez S, N.[4], Gonzales-Nilo, D.[2], Holmes, D.S.[1,3], Pohl, E.[5], Quatrini, R.[1,3]. Center for Bioinformatics and Genome Biology, Fundación Ciencia para la Vida, Santiago, Chile[1], Centro de Bioinformática y Simulación Molecular, Universidad de Talca, Talca, Chile[2], Facultad de Ciencias Biológicas, Universidad Andrés Bello, Santiago Chile[3], Laboratório Nacional de Luz Síncrotron, Campinas, Brasil. [4], Durham University, Durham, UK[5].
Fur is an evolutionarily conserved metallo-regulator controlling the expression of many cellular processes in response to intracellular iron concentrations, which ultimately depend on extracellular iron levels. Acidithiobacillus ferrooxidans is a bacterium that obtains energy from iron oxidation while thriving at pH 1-2 in the presence of an unusually high concentration of soluble iron. A. ferrooxidans encodes Fur (FurAFE) that we have shown earlier (1) to be a proficient iron responsive transcriptional regulator (2) to interact as a homo-dimer with a 19 base pair operator and (3) to control the expression of genes involved both in iron oxidation and iron homeostasis in this model bacterium.We have previously predicted the tertiary structure of FurAFE by homology modeling and suggested the spatial localization of key residues potentially involved in metal binding to gain mechanistic insights into the dilemma of how Fur can be involved in the regulation of iron uptake for metabolism as well as iron oxidation for energy production. High resolution biophysical tools, including EXAFS and ICP-MS analysis demonstrate the unusual result that the monomer form binds four iron atoms and one zinc. These results, combined with detailed sequence analysis, reveal conserved regions and residues responsible for metal recognition that pave the way towards understanding of the regulatory dilemma.
Acknowledgments: FONDECYT 1090451, 1100887, CONICYT Basal PFB16 and University Durham-Santander Scholarship.

81.Site-directed mutagenesis of extracellular cysteine residues in the sodium-coupledascorbic acid transporter-2 (SVCT2). **Sweet, K.**[1], Aylwin, C.[1], Salas-Burgos, A.[1], Rivas, C.I.[1], Vera, J.C.[1]. Departamento de Fisiopatología, Facultad de Ciencias Biológicas, Universidad de Concepción, Chile.[1].
The analysis of the structure-function relationship in the sodium-coupled ascorbic acid transporter-2 (SVCT2) is limited by the lack of information about its 3D structure. This transporter is a highly hydrophobic protein composed of 14 transmembrane segments, a characteristic associated with the NAT family of nucleobase-ascorbate transporters, with both the N- and the C-termini facing the cytoplasm. Given the difficulty of obtaining the crystal structure for this transporter and, in general, for members of the NAT family, alternative methods for obtaining structural and dynamic information can be used, being one of these methods cysteine scanning mutagenesis. One of the difficulties of conducting such studies is the presence of multiple cysteine residues in the primary structure of the protein and the possibility that these residues are essential for its activity, which is why it is important to determine the importance of these residues for the function of the SVCT2 transporter. We evaluated the role of the exofacial cysteine residues on SVCT2 function by replacing them with alanine or valine by site-directed mutagenesis, followed by expression in HEK-293 cells and analysis of subcellular localization and characterization of their functional properties. Our results showed that all mutants were efficiently expressed in HEK-293 and localized to the plasma membrane. Moreover, transport studies revealed that the mutant proteins are active in transporting ascorbic acid, and possess functional characteristics similar to native SVCT2, indicating that exofacial cysteine residues are not essencial for SVCT2 function.Project FONDECYT 1090501.

82.Site-directed mutagenesis of exofacial amino acid residues involved in substrate binding in the ascorbic acid transporter SVCT1. **Sepulveda, M.A.**[1], Haensgen, H.[1], Salas-Burgos, A.[1], Rivas, C.[1], Vera, J.C.[1]. Departamento de Fisiopatología, Facultad de Ciencias Biológicas, Universidad de Concepción, Chile[1].
The crystallization of lactose permease (LacY) was an advance for the study of transporters of the Major Facilitator Superfamily (MFS). Homology and conservation studies had allowed, accurately, set a common folding for its members like sodium-coupled ascorbic acid transporter-1 (SVCT1). Lately, researches revealed that the 12 transmembrane domain structure, with N and C termini oriented to the citoplasmatic face, tipically a MFS conformation, was also a signature of the Nucleobase-ascorbate transporter family (NATs), but, unlike members of the MFS, no symmetry of the two halves of the protein is observed, that explains certain inconsistencies with the SVCT1 3D-model based on LacY. We evaluated whether residues serine80, serine335, and asparragine478 in helix II, VII and XI respectively, proposed based on LacY 3D-model, are part of the exofacial substrate-binding domain in SVCT1. These aminoacid residues where individually replaced by site-directed mutagenesis. Mutant proteins were expressed in HEK-293 cells followed by assessing their subcellular localization by confocal microscopy and characterizing their functional properties by ascorbic acid transport experiments. The mutant proteins were sorted to the plasma membrane, however, there were important variations in their functional parameters. We found mutants showing a markedly decreased transport activity and mutants showing a major increase in the transport Km. Overall, our data are consistent with the role of the different transmembrane helices in the 3D-model of SVCT1 obtained by structural homology using the 3D-cristal structure of a member of the NAT family.Proyecto FONDECYT 1090501.

83.VvSDH1, a grapevine (Vitis vinifera) protein with homology to a sorbitol dehydrogenase from apple (Malus x domestica), oxidises sorbitol in vitro. **Araya, J.**[1], Tang, Y.[1], Handford, M.[1]. Laboratorio de Biología Molecular Vegetal, Departamento de Biología, Facultad de Ciencias, Universidad de Chile[1].
Sorbitol is the main product of photosynthesis and the form in which carbon is translocated via the phloem in species belonging to the Rosaceae family, which includes peaches (Prunus persica), pears (Pyrus spp) and apples (Malus x domestica). Once in the carbon sink organ, 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 (Solanum lycopersicum), soya (Glycine max) and maize (Zea mays), 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 from grapevine (Vitis vinifera) which we have named VvSDH1. VvSDH1 possesses all the molecular characteristics and the conserved domains present in the SDHs of other species. In silico analysis reveals that VvSDH1 is expressed differentially during the development of grape berries. In order to determine its enzymatic activity, VvSDH1 was cloned into plant binary vectors under the control of two different promoters. In order to test the functionality of these constructs, tobacco leaves and tomato fruits were transiently transformed using Agrobacterium tumefaciens. The results show that VvSDH1 is a cytosolic protein which is able to oxidise sorbitol in the presence of NAD+ in vitro. Funding: Innova 07CN13PBD-19.

84.Modular analysis of the Ribokinase family of enzymes. **Villalobos, P.**[1], Cabrera, R.[1], Baez, M.[1], Babul, J.[1]. Laboratorio de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Chile, Santiago.[1].
The ribokinase family includes ATP-depent sugar kinases with a broad range of specificities. The ribokinase fold is composed by a large domain, with a central β-sheet surrounded by α-helices, and a minor domain with four stranded β-sheets. However, the elements of secondary structure show a high structural diversity making it difficult to obtain good sequence alignments from structural superpositions. In order to overcome this difficulty, the complete non redundant set of 32 members found in the PDB was divided into modules. A module is a segment of contiguous residues that represents a compact region in the three-dimensional structure. The modules were identified by calculating the mean square distances between residues in a window covering a fixed number of residues, allowing to define the boundaries between two local minima of the distance function profile. The analysis resulted in the decomposition of each structure in ten conserved modules across the family. This analysis improves the sequence alignment compared to that obtained when the entire structure of family members was used. On the other hand, relevant residues common to the ribokinase family fold were identified by searching for those residues included within the atomic interaction network, but that present a low solvent accessibility in the structure. This analysis may be useful to improve protein sequence alignments among structures with high structural diversity (Fondecyt 1090336).

85.The First Full Model of Glycine Receptor. Sepúlveda-Ugarte, J.[1,2], Aguayo, L.[2], Bunster, M.[1]. Laboratorio de Biofisica Molecular, Depto. de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción[1], Laboratorio de Neurofisiología, Depto. de Fisiología, Facultad de Ciencias Biológicas, Universidad de Concepción[2].
Glycine receptors (GlyRs) play a critical role in neuronal excitability in the mammalian brain stem and spinal cord associated to sensory information, motor control and respiration, functions significantly altered during ethanol intoxication. The glycine-activated Cl- current can be potentiated by low, clinically relevant ethanol concentrations in central neurons and heterologous expression systems in unknown mechanism. GlyRs are pentameric ionic channel with similar topology subunits characterized by a large N-terminal extracellular domain (ECD), a transmembrane domain (TMD) with 4 transmembrane (TM1-4), an intracellular domain (ICD) connecting the TM3-4, and a small extracellular C-terminal domain. Residues present within TM2-3 were postulated for ethanol binding site. However, these residues mutations significantly interfered with the GlyR physiological properties by an intrinsic alteration in channel gating. On the other hand, our studies revealed that alpha1GlyR is modulated by G proteins through G?? heterodimers via basic residue domain (316RFRRK and 385KK) within the ICD and we shown that ethanol can modulate GlyRs through G protein activation and the degree GlyR/G?? functional interaction degree is critical for glycine-activated current ethanol-induced potentiation. Even we found that ethanol effects on the GlyR could be blocked by an ICD-derived peptide that interferes with GlyR/Gbetagamma interaction. We postulated the discrete molecular determinants existence for ethanol sensitivity within ICD structure. Unfortunately, there is no ICD structural information. Here we present ICD circular dichroism studies that shown a high alpha-helix content and the first full glycine receptor homology model, that included this domain. .

86.Determination of the binding orientation of the primer removal substrate in HIV-1 RT. Nogales, M.I.[1], Vargas, D.A.[1], León, O.[1]. Departamento de Virología, ICBM, Universidad de Chile.[1].
The reverse transcriptase (RT) is a heterodimer composed of p66 and p51 subunits and catalyzes the reverse transcription of the HIV-1 genome, an essential step in the retrovirus replication cycle. This enzyme has two activities: DNA polymerase (RNA and DNA dependent) and RNase H (hydrolysis of RNA). Recently studies shows that RT binds in two possible orientations to the substrate and the orientation depends on the composition of the substrate (RNA:DNA versus DNA:DNA). tRNAlys3 is the primer for the synthesis of the first strand of DNA in reverse transcription, then the RNase H removes the tRNAlys3 primer by a specific cut that defines the LTR termini for the integration. In this work we propose the use of crosslinking to determine the orientation of a substrate that mimics the reverse transcription intermediate for the removal of tRNA primer, on the RT-substrate complex. For this purpose, substrates modified in four different positions were used to couple the cross-linker disuccinimidyl glutarate (DSG) and reacted with wild type reverse transcriptase. We found that yield of cross-linking was different for each substrate and crosslinking of p66 or p51 within the heterodimer was dependent on the position of the modification. The crosslinking yield is sufficient for peptide sequencing to identify the crosslinked lysine to determine the orientation of the substrate in the RT with the tRNAlys3 primer.

87.Caveolin-1-mediated inhibition of the unfolded protein response in vitro and in vivo is linked to tumor suppression in a melanoma model. Diaz Morales, M.I.[1], Sanhueza, C.[1], Nuñez, S.[1], Rodriguez, D.[1], Lobos , L.[1], Quest , A.[1], Hetz , C.[1]. Laboratorio de Comunicaciones Celulares y Laboratorio del estrés celular y biomedicina, Centro FONDAP de Estudios Moleculares de la Célula (CEMC), Facultad de Medicina, Universidad de Chile[1],
Introduction: Caveolin-1 is a scaffolding protein associated with membranous structures: Plasma Membrane, Golgi and Endoplasmic Reticulum (ER). Caveolin-1 tumor suppressor functions are linked to target protein inhibition at cell surface. In tumors, the adaptive response: unfolded protein response (UPR), is triggered in ER. The possibility that Caveolin-1 functions in ER rather than cell surface may be relevant to tumor suppression is an unexplored research area.Methods: B16F10 mouse melanoma cells expressing or not Caveolin-1 were exposed to ER stressors tunicamycin and hypoxia. Changes in UPR markers were evaluated by PCR and Western blotting. Similar experiments were conducted in MDA-MB-231 breast cancer cells where endogenous Caveolin-1 expression was reduced using sh-RNA. Co-localization of Caveolin-1 with UPR markers was evaluated by immunofluorescence. For in vivo experiments, B16F10 cells were subcutaneously injected Into C57BL6 mice. UPR markers were evaluated after tumor formation. Results: Induction of UPR markers was attenuated in B16F10 and MDA-MB-231 cells expressing Caveolin-1 exposed to tunicamycin or hypoxia. Tumor suppression observed in C57BL6 mice for B16F10 cells expressing Caveolin-1 correlated with an attenuated UPR. Co-localization of Caveolin-1 and ER markers in B16F10 cells was significantly increased after tunicamycin. Conclusions: Caveolin-1 presence in tumor cells reduced UPR responses both in vitro and in vivo. Increased presence of Caveolin-1 in ER upon triggering UPR, suggests that Caveolin-1 may interact directly with UPR sensors. Subdued UPR responses in cells expressing Caveolin-1 are likely to be important for tumor suppression by this protein.

88.Rol of Cochaperone Bag3 in the autophagy activated by ER stress. Rodríguez, A.[1], Lavandero, S.[1]. Centro FONDAP Estudios Moleculares de la Célula, Facultad de Ciencias Químicas y Farmacéuticas/Facultad de Medicina, Universidad de Chile.[1].
Bag cochaperones are nucleotide interchange factors for Hsp/Hsc70 chaperones. They allow the selection of misfolded proteins and guide them towards different degradation systems. Bag1 destines substrates to proteasome pathway whereas Bag3 does to the autophagy process. Autophagy is activated in response to endoplasmic reticulum (ER) stress, a condition where misfolded proteins are accumulated inside the ER. Whether Bag3 participates in the activation of autophagy remains unexplored. To evaluate this possibility, HeLa cells were treated with tunicamycin for 1, 4, 8 and 24 h to induce autophagy. Bag1, Bag3 and LC3 levels were determined for Western blot in total cell lysates. The subcellular distributions of Bag3, PDI and LC3 were studied by immunocytochemistry in permeabilized cells. Bag3, Bag1 and Beclin1 were silenced in HeLa cells using shRNA encoded in lentiviral vectors. The results showed that tunicamycin treatment induced a transient decrease of Bag3 total levels (both in 86 and 45 KDa isoforms). This stressor also stimulated Bag3 relocation to the membrane fraction, positive for the ER marker PDI. Bag3 silencing decreased LC3II/LC3I basal ratio and increased Bag1 levels. The Bag1 silencing increased the number of autophagic cells. In addition, the Beclin1 silencing increased Bag3 total levels. In summary, these findings suggest that ER stress dependent autophagy is regulated by Bag3, establishing a connection with Hsp/Hsc70 chaperone system. AR holds a PhD fellowship from CONICYT. FONDAP 15010006.

89.Relationship between phosphorylated state of muscle glycogen synthase and GSK3beta,and hexoses availabilty in yhe seminal plasma in mammals. Villarroel-Espíndola, F.[1,2], Rodríguez-Gil, J.E.[2], Siebe, J.C.[1], Concha, I.I.[1]. Instituto de Bioquímica y Microbiología, Universidad Austral de Chile[1], Unitat de Reproducció Animal, Universitat Autónoma de Barcelona, España[2].
Glycogen is the main source of glucose and energy to sustain multiple biological processes. The sperm are cells that require high energy demand during ejaculation and to support its passage through the female tract to the egg. Glycogen synthase (GS), has been previously described in sperm and has the ability to synthesize glycogen. Depending on the species and reproductive strategy, the type of sugar present in seminal plasma may vary, and the availability of glucose-6-phosphate (Glc-6-P), allosteric activator of GS. The aim of this study was to compare the phosphorylation state of the muscle isoform of GS (MGS) and its kinase, GSK3beta, thereby linking the availability of seminal hexoses with glycogen synthesis. We took sperm from horse, pig, dog and human, and, by immunofluorescence and Western blot analysis, we detected high levels of inactive-GSK3beta (phospho-Ser9) in the four species, which was correlated with low immunodetection of inactive-MGS (phospho-Ser640). In addition, we evaluated by Western blot analysis the isoforms of hexokinase (HK) present in these models. We observed type I and III hexokinases in all sperm species. These results suggest that the type of sugar in the seminal plasma does not limit the availability of Glc-6-P and thus the activation of MGS. (CONICYT F.Villarroel; CONICYT AT-24100011; MECESUP UCO-0606; FONDECYT-1110508; Graduate school and DID-UACh).

90.Protein Kinase C delta (PKCδ) is required for re-expresion of Caveolin-1 induced by the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (4β-TPA) in colon adenocarcinoma cancer cells. Huerta, H.[1], Diaz, N.[1], Leyton, L.[1], Quest, A.[1]. Centro Fondap de Estudios Moleculares de la Célula (CEMC), Universidad de Chile[1].
Introduction: Caveolin-1 (Cav-1) is a generically expressed scaffolding protein that has been implicated in cancer as a tumor suppressor. However, during the cancer progression, Cav-1 may be re-expressed and favor the acquisition of malignant characteristics, such as multidrug resistance and metastasis. Interestingly, possible mechanisms involved in the re-expression of Cav-1 remain unclear. Here the possible role of pathways induced by the tumor promoter 4β-TPA in colon cancer cells, were analyzed. **Materials and methods:** The human colon adenocarcinoma cancer line DLD1, expressing low endogenous Cav-1 levels was evaluated. PKCδ was silenced using a short hairpin RNA and the effect on Cav-1 expression following stimulation with 4β-TPA, a known PKC activator was assessed. Activity of the transcription factors PPARγ and NFκB was measured by reporter assays. Cell migration was assessed in the transwell assay. **Results:** Silencing of PKCδ suppressed 4β-TPA-induced Cav-1 expression. In addition, the activity of PPARγ and NFκB, two transcription factors that control the expression of Cav-1, was lower in cells with reduced PKCδ levels. Importantly, the enhanced capacity of 4β-TPA stimulated cells to migrate was reduced in cells lacking PKCδ. **Conclusion:** 4β-TPA treatment increased the levels of Cav-1, as well as the activity of PPARγ and NFκB in a PKCδ-dependent manner, also increased PKCδ-mediated Cav-1 expression favored migration of these adenocarcinoma cells. These results link increases in Cav-1 triggered by the tumor promoter 4β-TPA to acquisition of a more malignant cancer cell phenotype.

91.Role of histidine 208 of Moloney murine leukemia virus (Mo-MLV) integrase in enzyme catalysis. Fuentes, Y.[1], Castillo, A.[1], León, O.[1]. Programa de Virología, ICBM, Facultad de Medicina,Universidad de Chile.[1].
Integrase (IN) is an essential enzyme for the replication cycle of retroviruses. IN catalyzes the 3'-proccesing viral DNA and integration of this DNA into host genome. The structure of retroviral integrases show three domains, an N-terminal domain (NTD), a central catalytic domain (CCD) and a C-terminal (CTD). The CCD domain contains a loop with a high degree of flexibility, where some residues interact with the viral DNA. In Mo-MLV IN the loop comprises residues 208 to 218. In our laboratory showed that replacing histidine 208 with alanine inactivates the enzyme. In order to study the role of H208 in the catalytic activity of IN, several substitutions were constructed by site-directed mutagenesis: H208N, H208R, H208G and H208E. IN mutants were purified and analyzed for 3'-processing, disintegration and concerted integration. We found that H208E substitution decreased concerted integration and disintegration. In contrast, H208R substitution maintains concerted integration. On the other hand substitutions H208N and H208G favored 3'-processing. These results indicate that His208 is not an essential residue for catalysis. However we found that some mutations had a different effect on 3'-processing and concerted integration, in agreement of the existence of two conformations of the enzyme during these reactions. It is possible that the flexible loop is involved in this conformational transition.

92.Development of an enzyme-linked immunosorbent assay analytical platform for determination of IgM specific anti-ISAV. Sandoval, R.[1], Olavarría, V.[1], Yáñez, A.[1]. Universidad Austral de Chile[1].
Infectious Salmon Anaemia is an Atlantic salmon emerging disease of Salmo salar caused by virus ISA. In this work we are developing an enzyme-linked immunosorbent assay (ELISA) for diagnosis of response of ISAV-vaccinated salmon. For this purpose were used anti-ISAV antibodies purified from egg yolk of hens immunized and also serum of immunized rabbit with proteins of the virus. To develop and standardize the best conditions of this methodology, in the early stages, we evaluated the antibodies by indirect ELISA using anti-ISAV antibodies present in rabbit serum at a dilution range 1:500 - 1:2500. The signal was comparable to the positive control, which used a monoclonal anti-hemagglutinin. Interestingly, the best results were achieved when we perform two changes: heat inactivation of rabbit serum and the use of milk instead of BSA in the blocking stage. Finally, we performed an indirect ELISA assay with sera from fish infected with ISA virus, however the results were not expected because the signal was slightly higher than the negative control, presumably by the low titer virus-specific IgM in serum of fish tested. The results of this study show a preliminary immunodetection of ISAV by indirect ELISA method however, requires the use of complementary methods that allow chromatographic purification and concentration of IgM serum of fish before testing. This work was supported by FONDEF D08I1055.

93.Production and characterization of chicken antibodies against ISA virus as a diagnostic tools. Sandoval, R.[1], Olavarría, V.[1], Romero, A.[1], Yáñez, A.[1]. **Molina, A.[1]**. Universidad Austral de Chile[1].

The infectious salmon anemia virus (ISAV) is a disease that primarily affects Atlantic salmon (Salmo salar) caused high mortality in Norway, Scotland, Canada, the United States and Chile. The virus is classified in the Orthomyxoviridae family and is the only member of the genus Isavirus, its genome consists of 8 segments of linear single-stranded RNA with negative polarity, which can encode up to 11 polypeptides. Among the proteins of importance is the hemagglutinin esterase (HE) which allows endocytosis of the virus and its class is a marker of variability to have a highly polymorphic region (HPR). Given the importance of the virus in the salmon industry, we set the goal of obtaining and characterization of IgY immunoglobulins against ISAV proteins purified from egg yolk of hens immunized with the proteins. This provides a valuable diagnostic tool for monitoring the virus. To obtain antibodies chickens were immunized with the protein stain, band of 40 kDa and 80kDa isolated by SDS-PAGE. The IgY were purified by the method of precipitation with ammonium sulfate and its specificity was tested by drop assay, western blot and IFAT, as well as being compared to a monoclonal antibody against the ISA virus HE. The purified antibodies showed a low degree, but with good specificity against ISAV demonstrating it useful to diagnosis. This work was supported by FONDEF D08I1055.

94.The PBS, DIS And SD domains of the HIV-1 5`UTR play a role on the activity of the HIV-1 IRES. **Carvajal, F.[1]**, Vallejos, M.[1], Pino, K.[1], López-Lastra, M.[1]. Laboratorio de Virología Molecular, Centro de Investigaciones Médicas, Facultad 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 protein. Unlike many other viral IRESs, activity of the HIV-1 IRES is not exclusively determined by its secondary structure, it also relies on RNA interaction with cellular proteins. In order to better understand the role of well defined structures present within the viral 5`UTR, natural variants of the 5`UTR of HIV-1 were isolated from clinical samples and the presence of an IRES was evaluated. Compared with the reference 5`UTR sequence (pNL4-3-clone), variants revealed nucleotide differences mostly located in single-stranded regions of the secondary structure model of the 5`UTR. These data suggest that the overall secondary structure of the leader is not altered by these nucleotide changes. *Ex-vivo* assays using bicistronic constructs showed that despite of the important number of nucleotide changes incorporated in the variant 5`UTRs, all exhibit IRES activity. Based on their activities, the variant IRESes were divided in three groups, the first group exhibited comparable IRES activity to the reference 5`UTR. The second group exhibited enhanced activities, sharing some nucleotide changes in the PBS and DIS domains of the 5`UTR, and the third group exhibited lower activities, sharing some nucleotide changes in the PBS and SD domains. These results suggest a regulatory role of the PBS, DIS and SD domains in the activity of the HIV-1 IRES. Work supported by FONDECYT 1090318.

95.Proteins N and NSs of Andes hantavirus participates in the S segment mRNA translation efficiency. **Castillo Vargas, E.[1]**, Vera Otarola, J.[1], López-Lastra, M.[1]. Laboratorio de Virología Molecular, Centro de Investigaciones Médicas, Facultad de Medicina, Pontificia Universidad Católica de Chile[1].

The S segment mRNA (SmRNA) of Andes virus (ANDV) encodes the nucleocapsid (N) protein. Recent studies from our group showed the expression of a second protein encoded by an overlapping ORF (+1), the ANDV non-structural protein NSs. Reports show that the hantavirus N protein participates in translation initiation of SmRNA. The function of the NSs protein in translation of the SmRNA has not been studied. For this reason, and taking in consideration that knowledge regarding the function of the ANDV S segment encoded proteins is scarce, in this work we evaluated the role of the ANDV N and NSs proteins on the translation of the ANDV SmRNA. For this purpose, we generated two SmRNA-like constructs, N-RNA and NSs-RNA, either having the N or the NSs initiation codon in frame with a reporter gene. The ex-vivo results show that the ANDV N and NSs proteins differentially modulate the translation of the ANDV SmRNA. Whilst the N protein exhibit a stimulatory effect over both N-RNA and NSs-RNA constructions, the NSs protein only stimulates, in a concentration dependent manner, translation from the NSs-RNA. Therefore, results suggest that both ANDV proteins N and the NSs display RNA chaperone activity, participating in the SmRNA translation process. CONICYT Scholarship. Funding by Instituto Milenio de Inmunología e Inmunoterapia (IMII), FONDECYT 1100756 y NIH/International Collaborations in Infectious Disease Research (ICIDR)/Fogarty (2U01AI045452-11).

96.Transcriptome analysis of *P. purpurogenum* by means of next-generation sequencing technology and differential expression of lignocellulolytic genes in different carbon sources. **Mardones, W.[1]**, Klagges, C.[1], Eyzaguirre, J.[1]. Universidad Andrés Bello[1].

Lignocellulosic biomass (main component of plant cell wall) is a renewable energy source and its mechanisms of degradation are of interest because of possible biotechnological applications. Filamentous fungi are capable of regulating the expression of lignocellulolytic enzymes depending on the composition of the carbon source used for growth, thus allowing for their efficient degradation. Carbon source from natural substrates, such sugar beet pulp, induce the expression of these enzymes. Rather simple and soluble carbon sources, like glucose, repress their expression. *P. purpurogenum* degrades lignocellulose carbon sources such as sugar beet pulp (rich in pectin and cellulose) and secretes enzymes needed for its breakdown. The study of these enzymes is a relatively unexplored subject, mainly due to laborious work required for their identification by the classical techniques of biochemistry. The development of technologies for next-generation genome sequencing allow the identification of the transcripts generated in a given growth condition (RNA-seq). This offers a valuable tool for the analysis of the expression in non-model organisms. In this work, using second-generation sequencing, we have identified a large number of genes expressed when *P. purpurogenum* grows on glucose or sugar beet pulp as carbon source. Several of these genes encode for hypothetical lignocellulolytic enzymes; some of them are differentially expressed under these two culture conditions, as demonstrated by qPCR. Financial support: FONDECYT 1100084 and UNAB DI-03-10/R.

97.Identification of functional cis-regulatory elements in intron 5 of *runx1* gene. Fernández, V.[1], Rebolledo, B.[1], Martínez, M.[1], Gutiérrez, S.[1]. Laboratorio de Regulación Transcripcional y Leucemia, Departamento de Bioquimica y Biología Molecular, Universidad de Concepción [1].

RUNX1 is a crucial hematopoietic transcription factor that belongs to the RUNX family of transcription factors, which share homology in a 128aa DNA binding domain. The *RUNX1* gene is the most frequently targeted gene in chromosomal translocations in acute myeloid leukemia (AML) with the t(8;21) translocation accounting for up to 12% of AML-M2 patients. All the DNA breakpoints mapped until now are clustered in intron 5 of this gene. Interestingly, this intron exhibit several chromatin structural elements, such as DNase I hypersensitive sites, hyperacetylation of histone H3 and enrichment in the enhancer-related histone modification H3K9/14Ac, associated with active chromatin and presence of transcriptional regulatory elements. This particular chromatin organization suggests that biologically relevant DNA sequences maybe located in this intron. To test this hypothesis, we performed an *in silico* analysis to identify conserved non-coding sequences (CNS). Using a comparative genomics approach, we identified nine CNS in intron 5 of the *RUNX1* gene with high probability to be cis-regulatory elements of transcription. Of the putative regulatory sequences, CNS-T2 has the highest conservation between human-mouse-rat (77% identity); furthermore it is located near a H3K9/14Ac enriched region. Therefore, to evaluate the activity of this region *exvivo* we cloned it in luciferase reporter vectors under the control of SV40 and *RUNX1* promoters. Our results show that CNS-T2 acts as transcriptional regulatory element in a position- and distance- independent manner. Taken together our results demonstrate that CNS-T2, acts as a transcriptional cis-regulatory element. FONDECYT1100670.

98.Analysis of hSWI/SNF nucleosome remodelling activity under recruitment by estrogen receptor alpha. Fernández García, Y.[1], Alarcón, V.[1], Gutiérrez, J.[1]. Laboratorio de Regulación Transcripcional, Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile[1].

Eukaryotic organisms have their nuclear genetic material organized into protein-DNA associations that form structures known as nucleosomes, which are repeated and grouped giving rise to the chromatin. Beyond its structural role, chromatin participates in the regulation of fundamental processes at the DNA level. Access to the promoter regions of genes by the transcriptional machinery of the cell is one of the main barriers of gene expression and is restricted due to the dense arrangement of the chromatin. The assembly of the RNAPII on gene promoters is orchestrated by various transcription factors. These possess DNA binding domains which identify specific sequences. They also have regions that allow the interaction with other proteins or protein complexes, which implies that they can recruit several entities to particular sites in the genome. Among the protein complexes that can be recruited are those with the ability to remodel chromatin, been the SWI/SNF family one of them. This family of complexes uses the energy from ATP hydrolysis to mobilize nucleosomes. In order to evaluate the influence of transcription factors on the *in vitro* activity of SWI/SNF complexes in mammals, nucleosome remodeling assays were preformed in the presence of hERalpha. It was shown that this nuclear receptor has the ability to recruit hSWI/SNF to target sequences and concentrate their sliding activity in a precise zone. Our results suggest that BAF subcomplexes of the hSWI/SNF family do not cause eviction on the nucleosome to which it is recruited.FONDECYT-1110803.

99.Human CCAAT/Enhancer-binding protein beta (C/EBPbeta) interacts with chromatin remodelling complexes of the imitation switch (ISWI) subfamily. Valenzuela, N.[1], Del Río, V.[1], Hepp, M.[1], Fernández, Y.[1], Gutiérrez, J.[1]. Laboratorio de Regulación Transcripcional, Departamento. de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile.[1].

The transcription factor C/EBPbeta is involved in several cellular processes, such as proliferation, differentiation and energy metabolism. This factor exerts its activity through recruitment of different proteins or protein complexes, including the ATP-dependent chromatin remodeling complex SWI/SNF. The C/EBPbeta protein is found as three major isoforms, C/EBPbeta1, 2 and 3. They are generated by translation at alternative AUG initiation codons of a unique mRNA, being C/EBPbeta1 the full length isoform. It has been found that C/EBPbeta1 participates in terminal differentiation processes. Conversely, C/EBPbeta2 and 3 promote cell proliferation and are involved in malignant progression in a number of tissues. The mechanisms by which C/EBPbeta2 and 3 promote cell proliferation and tumor progression are not fully understood. In our present work we sought to identify hC/EBPbeta interacting proteins using a proteomic approach. We found that all three isoforms interact with hSNF2H and hACF, components of ACF and CHRAC chromatin remodeling complexes, which belong to the Imitation Switch (ISWI) subfamily. Additional protein-protein interaction studies confirmed this finding, which also shown that hC/EBPbeta directly interacts with hACF1. By overexpressing hC/EBPbeta, hSNF2H and hACF1 in HepG2 cells and analyzing variations in expression of cyclin D1 and other C/EBPbeta target genes, we observed a functional interaction between C/EBPbeta and SNF2H/ACF1, characterized mainly by suppression of C/EBPbeta transactivation activity in the presence of SNF2H and ACF1.FONDECYT 1085092.

100.Isolation and characterization of grape *MYB4* homologues involved in the regulation of flavonoid synthesis in grapevine (*Vitis vinifera* L.) Loyola, R.[1], Matus, J.T.[2], Walker, A.R.[3], Arce-Johnson, P.[4]. [1]Departamento de Fruticultura y Enología. Facultad de Agronomía e Ingeniería Forestal. Pontificia Universidad Católica de Chile, Santiago, Chile [1], [2]CRAG-Centre de Recerca Agrigenomica, Consorcio CSIC-IRTA-UAB, Barcelona, Spain[2], [3]CSIRO Plant Industry and CRC for Viticulture, Glen Osmond, Australia[3], [4]Departamento de Genética Molecular y Microbiología. Facultad de Ciencias Biológicas. Pontificia Universidad Católica de Chile, Santiago, Chile[4].

Flavonoids are the most important molecules responsible of colour, flavour and aroma in the grape berry. Flavonoid synthesis arises from the phenylpropanoid pathway. Its key enzymes are tightly regulated by the combinatorial interaction of MYB, bHLH and WDR transcription factors, as found in many plant model species as well as in grapevine. Several members of the MYB Superfamily regulate different steps of the phenylpropanoid pathway. Some of these positively regulate the accumulation of phenolic compounds and other repressing. In Arabidopsis, a group of six MYB proteins (MYB3, MYB4, MYB6, MYB7, MYB8 and MYB32) belonging to subgroup 4 (C2 repressor motif clade) regulate of primary and secondary metabolism at different stages of plant development and in response to diverse stimuli by repressing. AtMYB4 controls sinapate esters biosynthesis in a UV-dependent manner. Others MYB proteins are involved in the control of cell wall composition. In grapes, we have identified two flavonoid-related MYB repressor genes (VvMYB4a and VvMYB4b) controlling flavonoid accumulation. Interestingly, phylogenetic analyses revealed that genes grouped together with AtMYB4. Suggesting that possibly would be involved in similar functions. We will present new MYB genes controlling flavonoid accumulation in grape, such as VvMYB4 repressor, and discuss their importance in this network. The analysis of these genes will provide the basis for field and biotechnological approaches in order to improve wine potential by manipulating these fruit metabolic pathways. **Acknowledgements:** Fondecyt 1100709, Innova Corfo 07Genoma01, Millennium Nucleus in Plant Functional Genomics.

101.Glucose-induced production of a *Penicillium purpurogenum* xylanase by *Aspergillus nidulans*. **Ravanal, C.**[1], Rosa, L.[1], Vaca, I.[2], Chávez, R.[3], Eyzaguirre, J.[1]. Universidad Andrés Bello[1], Universidad de Chile[2], Universidad de Santiago de Chile[3].

Xylan, the main polysaccharide from hemicelluloses, is degraded by an array of enzymes called xylanases. Xylanases have great biotechnological potential in food processing and production of biofuels and chemical feedstocks. Among them are the endoxylanases, which hydrolyze the main chain of xylan. The fungus *Penicillium purpurogenum* when grown on any of several carbon sources (oat spelts xylan, birch wood xylan, sugar beet pulp, etc.) secretes to the culture medium different endoxylanases, among them Xyl A and B. The heterologous secretion of endoxylanase B from *Penicillium purpurogenum* using glucose as inducer was achieved in Aspergillus nidulans. For this purpose, plasmid pEVXB, containing the xylanase B cDNA (including its own signal peptide) under the control of the glyceraldehyde-3-phosphate dehydrogenase promoter, was constructed and used to transform *A. nidulans*. Analysis of transformant clones showed that several of them secreted extracellular xylanase activity when grown in a medium containing glucose. The clone showing the highest xylanase activity was chosen for further work. When this clone was grown on glucose, xylanase activity (0.72 U/ml) was detected in the culture supernatant. This was confirmed by a zymogram analysis and the amplification of xynB cDNA from this clone. To our knowledge, this is the first example of the production of a xylanase from *Penicillium* in heterologous fungal hosts using glucose as inducer. This work was supported by FONDECYT (Grant 1100084), UNAB (Grant DI-03-10/R) and DICYT-USACH.

102.Characterization of the dormancy-inducible Mb1767 gene of Mycobacterium bovis. **Santibáñez, P.**[1], Palavecino, C.[2], Zárraga, A.M.[1]. Universidad Austral de Chile[1], Pontificia Universidad Católica de Chile[2].

Mycobacterium tuberculosis and Mycobacterium bovis, are the pathogens for human and bovine tuberculosis, respectively. Comparative genome analysis among bacilli shows a 99.95% of sequence identity and 2437 SNPs. In addition, 6% of the genes display different expression at the transcriptome level. Upon macrophage infection, the level of oxygen drops and the bacilli switches from a replicative to a non-replicative state (dormancy). It follows the increase of NO and induction of the dormancy regulon. The narK2 gene, which codes for a nitrite / nitrate antiporter, belong to this regulon. In M. bovis narK2 is inactive, thus suggesting alternative strategies for energy production. Divergent to narK2, the hypothetical Rv1738 is located. This gene is activated by y- IFN, what suggests a role during early infection. Rv1738 is not activated under NO exposure while its orthologous in M. bovis, Mb1767, is highly induced. Thus, different regulatory mechanisms appears to control Mb1767/Rv1738 and narK2 genes between the two species. In this work, the Mb1767 gene was characterized and the promoter activity determined using lacZ reporter vector. The in silico analysis showed that Mb1767 would code for a 10 kDa protein with three beta-chains and one alpha helix. The promoter region displayed four consensus regions for the binding of the transcriptional dormancy regulator DosR. Mutants at the DosR elements and the SNP, demonstrate that the SNP region is required for NO-induction of Mb1767. The involvement of Mb1767 gene during M. bovis survival is further discussed.

103.Differential effect on transcription of pituitary factors in estrogen treated carp. **Pérez, A.**[1], Valenzuela, G.[1], **Navarro, M.**[1], **Romero, A.**[2], **Figueroa, J.**[1], Kausel, G.[1]. Instituto de Bioquímica y Mirobiología, Facultad de Ciencias, Universidad Austral de Chile, Valdivia[1], Instituto de Patología Animal, Facultad de Ciencias Veterinarias, Universidad Austral de Chile[2].

Transcription factors play pivotal roles as central nodes in the network of gene expression regulation. With the aim to reveal estrogenic effects on pituitary factors, expression of a series of hormones and tissue specific as well as other homeo box containing transcription factors were analyzed in estrogen (E2) treated carp. Male carp were injected on three consecutive days with 0,5mg/kg E2 and sacrificed on the fourth day. Total RNA was prepared from pituitary and as control from liver samples. Specific transcripts were quantified by RT-qPCR in duplicates, normalized for beta-actin and fold-change calculated respect to control fish injected with vehicle only. All fish responded to E2 treatment as shown by significantly increased vitellogenin transcription in liver. Differential effects included increase of somatolactin, decrease of prolactin, no change of TSH-beta accompanied by diminished Pit1 and Rpx gene expression in pituitary, the master gland releasing secretions that control all other glands. These particular results might be due to the experiment performed on summer acclimatized fish and strongly suggests to take in to account specific environmental condition that might be reflected in epigenetic marks when analyzing biomarkers.

104.Effect of the copper concentration on the growth of *Phanerochaete chrysosporium* PcACE1 mutant lacking the FIFTH CYS motif. **Essus, K.**[1], Bajas, F.[1], Campbell, H.[1], Bull, P.[1]. Pontificia Universidad Católica de Chile[1].

Copper is a key element in living organisms. It plays a vital role as cofactor of metalloenzymes. Therefore, it is necessary to regulate its intracellular level. ACE1 is a Saccharomyces cerevisiae transcription factor 225 amino acids long, activated at high copper concentration and induces target genes (metallothionein, superoxide dismutase and cytochrome C oxidase). ACE1 contains 3 Cys motifs of the type C-X-C and C-X-X-C. The first one binds Zn, and the second and third bind copper. The ACE1 ortholog in Phanerochaete chrysosporium is PcACE1. It is 633 amino acids long and surprisingly, it has 5 instead of 3 Cys motifs. In this study we analyzed the role of the fifth motif on growth at different copper concentrations. We introduced by site-directed mutagenesis a stop codon between Cys motifs four and five in PcACE1. Both wild-type PcACE1 and the resulting mutant (PcACE1-MUT1) were cloned in the yeast expression vector p416GPD and transformed an ACE1Delta Saccharomyces cerevisiae mutant. Resulting clones were grown at copper concentrations between 1 and 400 microM and the absorbance $_{600nm}$ was determined for each condition as growth parameter. Preliminary results show that yeast ACE1Delta mutant does not grow at 50 microM $CuSO_4$; whereas wild-type PcACE1 is resistant to 100 microM $CuSO_4$. PcACE1-MUT1 is more resistant than ACE1Delta mutant but less than wild-type PcACE1. These results suggest that Cys motif five of PcACE1 is involved in the capacity to grow at high copper concentrations. Proyecto Límite 18-2010.

105.Regulation of the RUNX1 distal promoter activity by sequences in the 5'UTR region. Martínez, M.[1], Mella, J.[1], Javed, A.[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 Oral and Maxillofacial Surgery, University of Alabama, USA[2].

RUNX1, an essential regulator of hematopoiesis, belongs to the family of runt related transcription factors. RUNX1 expression is controlled by two promoters: the distal (P1) and the proximal (P2). Both promoters are separated by 160Kb and have different expression patterns, with P1 isoform been mainly expressed in T cells. In silico analysis of the P1 promoter identified putative RUNX binding sites both in the P1 promoter and the 5' untranslated region (UTR). Interestingly, two of the three RUNX binding sites located in the 5'UTR are conserved between rat and human and among other members of the RUNX family suggesting that they may have a regulatory role on P1 activity. To test this hypothesis, we cloned 600bp of the P1 promoter with or without 500bp of the 5'UTR, as well as the 500bp 5'UTR alone, upstream of the luciferase reporter gene. The P1 promoter shows four-fold higher activity than the P1+5'UTR or 5'UTR alone. Moreover, P1 promoter show a three-fold response to RUNX1 over-expression, which was completely absent in the 5'UTR and almost completely abrogated in P1+5'UTR. To validate that this inhibitory response is mediated by RUNX regulatory motifs in the 5'UTR, we perform site directed mutagenesis. Mutation of all three RUNX sites in the 5'UTR of the P1+5'UTR results in a transcriptional response to RUNX1 over-expression similar to the restores P1 promoter. In conclusion our data demostrate that the RUNX binding motifs located in the 5'UTR regulate P1 promoter activity.FONDECYT1100670.

106.Identification of PPRs factors required for mitochondrial transcript editing in Arabidopsis thaliana. Arenas, A.[1], Zehrmann, A.[2], Moreno, S.[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].

In higher plant mitochondria more than 400 RNA editing sites have been described. The determinants of specificity and deamination mechanism (C to U) are currently unknown. Recently, pentatricopeptide repeat (PPR) proteins, which form an expanded large gene family in higher plants (more than 450 members in Arabidopsis) have been shown to be essential factors for it. However, until now less than 10% of editing sites has been associated to PPR protein. In this work, we have characterized independent TDNA lines in four PPR genes, selected by their predicted mitochondrial destination and low similarity to other PPR proteins. The SNaPshot methodology was employed to analyze more than 350 editing site in mitochondrial transcripts of homozygous mutant and their wild type counterpart. For two insertional lines in PPR7170 and PPR6900 genes, we found the same editing level at all analyzed sites in mutant and wild type plants. In contrast, mutants in the PPR5060 and PPR3580 genes showed decreases in editing at specific sites. In the PPR5060 mutant we only found the nad1-308 site not edited, being the first PPR factor described for editing of nad1 transcript. Finally, the PPR3580 mutant present partial editing at four sites in four mitochondrial transcripts: cox2-320, ccb203-138, nad4-166 and cox3-311. Interestingly, the most affected sites: nad4-166 and cox3-311 present high similarity, which could be related with a strong defect detected at editing level. Our results reinforce the critical role of PPR factors to editing specificity determinant. FONDECYT-1100601,Núcleo-Milenio-P10-062-F,ApoyoTesis-CONICYT-24100161 .

107.Characterization of cis-acting elements involved in the expression of clock- and stress-regulated genes in *Neurospora crassa*. Stevens-Lagos, A.[1], González-Vogel, A.[1], Larrondo, L.F.[1]. Depto. Genética Molecular y Microbiología, Facultad Ciencias Biológicas, Pontificia Universidad Católica de Chile.[1].

Research in *N. crassa* has been instrumental to our current understanding of how the circadian clock works and how it interacts with the environment to sustain oscillations in gene expression over time. Despite the wealth of knowledge on the molecular basis of its central oscillator, little is known about how it can temporally control gene expression. As in other organisms, circadian control of transcription is widespread in *Neurospora*, with many of these clock-controlled genes also being stress-responsive. Metallothioneins (MT) are cystein-rich proteins of low molecular weight involved in metal storage and stress responses. The MT gene of *N. crassa* has been shown to respond to copper stress and was identified as the first non-developmentally clock- controlled gene in this fungus. Despite this, no *cis*-acting elements responsible for these responses have been identified in this gene. *con-10*, a developmentally-regulated gene, is also clock-controlled in *Neurospora*. This gene is also induced by light and stressful conditions. Despite the several inputs shown to control the expression of this gene, no function has been reported for *con-10* and the cis elements involved in its transcriptional regulation are unknown. In this work, we report the identification of *cis*-regulatory elements involved in the expression of both MT and *con-10* genes in *Neurospora*. Our results suggest that different promoter elements are responsible for their circadian regulation and responses to environmental cues, highlighting the complexity of transcriptional regulation in this model organism. Fondecyt 1090513, TWAS.

108.The iron homeostasis transcriptional network is associated with light and circadian regulation in the ascomycete *Neurospora crassa*. Muñoz, F.[1,2], Olivares-Yañez, C.[1,2], Catalán, V.[1,2], Larrondo, L.F.[1,2]. Depto. Genética Molecular y Microbiología, Facultad Ciencias Biológicas,[1], Pontificia Universidad Católica de Chile.[2].

Iron is an essential element for all microorganisms, including fungi. This metal participates in a wide range of biological processes, but in excess, iron could be dangerous to cells since it can produce ROS capable of reacting with key biomolecules. Consequently, a tightly regulated control mechanism of iron incorporation has evolved in most fungi. This system is a double negative transcription circuit between two repressors, one acting when iron is available in excess and the other when iron is depleted. These two transcription factors can repress each other in their respective conditions. In *Neurospora crassa*, recent studies have shown that SRE -the repressor acting under iron-rich conditions- is a direct target of the WC-1 photoreceptor/ transcription factor. In our lab, we have started the characterization of this iron/light transcriptional network in Neurospora. First, we have found that HapX is the repressor acting under iron depleted conditions. Mutants of *sre* and *hapx*, and *wc-1*, show differences in the transcription profiles (RT-qPCR) of putative target genes involved in the iron homeostasis when examined under different relevant environmental conditions (light and dark). Finally, since WC-1 is a key component of the circadian clock, the endogenous molecular timekeeper responsable of the organism?s synchronization to external inputs from -days and nights-changes, iron homeostasis regulation was analyzed as a potential output pathway of the clock. Interestingly, we observed an oscillatory expression pattern for the SRE transcript levels and several genes involved in iron regulation. Fondecyt 1090513, TWAS, .

109.miR-146a and miR-638 expression in triple negative breast cancer tumors. **Zavala, V.**[1], Herrera C.[1], Tapia T.[1], Cruz, A.[2], Segovia, L.[2], Alvarez, M.[3], Camus, M.[4], Carvallo, P.[1]. Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile[1], Hospital Barros Luco, Santiago, Chile[2], Clínica Las Condes, Santiago, Chile[3], Centro de Cáncer, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile.[4]

MicroRNAs are non-coding RNA sequences of about 22 nucleotides long that have been described to participate in cancer progression and treatment resistance. Triple negative breast cancer (TNBC) tumors correspond to a particular group characterized by being more aggressive, to have no specific therapy and a high percentage not expressing BRCA1. Lack of expression of BRCA1 may be caused by promoter hypermethylation, truncating mutations, or mRNA instability. In relation to BRCA1 mRNA regulation two miRNAs, miR-146a and mir-638, have been involved. We have previously described that BRCA1 promoter is hypermethylated, in 68% of breast cancer tumors. For this reason we sought for miR-146a and miR-638 changes of expression in those tumors, to determine the extent of the regulation of BRCA1 through miRNAs. Total RNA was extracted from formalin-fixed tumors (n=19). Then miRNAs expression was evaluated using qPCR-TaqMan assay and the results were analyzed through the REST software, determining miRNAs expression ratio in relation to a control RNA. Our results showed that 58% (11/19) of the analyzed tumors presented an increased expression of at least one of these miRNAs, and four of these have absent BRCA1nuclear expression. Tumors with normal nuclear BRCA1 expression showed no alteration of these miRNAs. To have a good correlation between miR-146a and miR-638, and BRCA1 expression we need to increase the number of breast tumors analyzed. Nonetheless this data suggest that miRNAs might regulate BRCA1 expression in TNBC, consisting in relevant actors of breast cancer progression. FONDECYT-1080595.

110.Evaluation of the expression of gene ZRT1 in conditions of nitrogen excess and limitation in Saccharomyces cerevisiae wine yeasts. **Contreras, A.**[1], Salinas, F.[2], Garcia, V.[2], Perez-Ortín, J.E.[3,4], Ganga, M.A.[2], Martínez, C.[5]. Departamento en Ciencia y Tecnología de los Alimentos. Universidad de Santiago de Chile[1], Departamento en Ciencia y Tecnología de los Alimentos[2], Laboratorio de Genómica Funcional de Levaduras. Universidad de Valencia. España.[3], [4], Departamento en Ciencia y Tecnología de los Alimentos y Centro de Estudios en Ciencia y Tecnología de los Alimentos. Universidad de Santiago de Chile[5]. Sponsored by E. Kessi.

Nitrogen is an essential nutrient for the adaptation and development of S. cerevisiae in wine fermentations, and thus it is important to obtain knowledge of the genetic factors related to its metabolism. Through global analyses we identified genes that varied their expression in strains that were genetically similar but which showed differences in nitrogen uptake. One of these was gene ZRT1, which codes for a zinc transporter and could be related to Gln3p, the main nitrogen metabolism regulator in yeast. To determine if ZRT1 is related to nitrogen uptake in this work we evaluated the expression of this gene in conditions of excess and nitrogen limitation. This was carried out using different wine strains and along different points of the growth curve. Results indicate that all strains show an important change in the expression of ZRT1 when going from one growth phase to another and the change is greater where there is an excess of nitrogen in the media. On the other hand, in majority of the strains an increase in the expression of this gene was observed under conditions of excess nitrogen, except for one strain which is characterized for presenting lower ammonium uptake with respect to the others. These results suggest a previously undocumented relationship between gene ZRT1 and the nitrogen uptake. Fondecyt 1100509, CONICYT scholarship for PhD studies, Scholarship in support of PhD Thesis AT24091033, Scholarship in support of research Universidad de Santiago de Chile.

111.Effect of Flumequin and Florfenicol on Expression in Metabolic Proteins and Drug Resistance Proteins in Head Kidney Cells of Atlantic salmon (SHK-1) cultured *in vitro*. **Barrientos, C.A.**[1], Aguilar, M.N.[1], Carreño, C.F.[1], Villalba, M.[1], Manríquez, R.A.[1], Castilla, S.M.[1], Calderón, A.F.[1], Yañez, A.J.[1], Cárcamo, J.G.[1]. Universidad Austral de Chile[1].

Salmonid culture in our country has been target to diverse pathologies, as also in the uncontrolled use of drugs such as antibiotics, which are in widespread use in aquaculture, not knowing the real effects in metabolism of the fish.The effect of two antibiotics commonly used in salmon farming, flumequine and florfenicol were evaluated on the expression levels of proteins involved in metabolism and drug resistance (MDRs) in SHK1 cells.The expression levels of proteins involved in drug metabolism (CYP1A, CYP3A, FMO and GST) and proteins involved in xenobiotic efflux transport (Pgp and MRP1), were measured by Western blot after flumequine and florfenicol treatments, at a concentration of 10 ug / mL in assays of 24, 48 and 72 hours. Cell viability was also measured by MTT assays.The results obtained showed significant fluctuations with respect to control in the levels of expression of all proteins analyzed in response to flumequine and florfenicol treatments, while no effect was observed on cell viability.These results show that in this salmonid cell line, the treatment with antibiotics changes the expression levels of protein metabolism and of drug resistance proteins, which may be associated with the development of drug resistance phenomena in subsequent drug therapy and induce drug interactions. Furthermore, this information allows further study of the mechanisms involved in cell metabolism and elimination of drugs in a productive sector of high economic relevance.Financiado por proyecto FONDEF D08I1096.

112.Cloning and heterologous expression of the alpha-amylase inhibitor alpha-AI1 in *Kluyveromyces lactis*. **Brain-Isasi, S.**[1,2], Álvarez-Lueje, A.[1], Corsini, G.[2]. Laboratorio de Farmacoquímica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile[1], Laboratorio de Bacteriología Molecular, Facultad de Medicina, Universidad Diego Portales[2].

Proteinaceous inhibitors of the enzyme alpha-amylase are widespread in plants. The seeds of several varieties of the common bean (*Phaseolus vulgaris*, L.) contain a plant defense glycoprotein called alpha-AI1, also known as phaseolamin, that inhibits the alpha-amylases of mammals and insects. Due to the above, this protein has a high therapeutic potential. Against this background, in this work we address the cloning and expression of alpha-AI1 in a *Kluyveromyces lactis* heterologous system. The alpha-AI1 codifying gene is encoded by a single locus in the genome of several varieties of *P. vulgaris*, so the alpha-AI1 gene was amplified by PCR from *P. vulgaris* cv. Hallado genomic DNA. This amplicon was ligated into a TOPO T/A based cloning plasmid for further transformation of *Escherichia coli* cells with it. The fragment with the alpha-AI1 codifying gene was released with the enzymes *Xho*I and *Eco*RI for the later ligation into the *K. lactis* expression vector pKLAC2. *K. lactis* positive clones for the alpha-AI1 gene presented an inhibitory activity against porcine pancreatic alpha-amylase. Acknowledgements: Conicyt Grant for Ph.D. Studies in Chile. Conicyt Grant for Ph.D.Thesis Development AT-24100168.

113.Sequential Establishment of Marks on Soluble Histones H3 and H4. Alvarez, F.[1], Muñoz, F.[1], Schilcher, P.[2], Imhof, A.[2], Almouzni, G.[3], Loyola, A.[1,4]. Fundación Ciencia para la Vida[1], Munich Center of Integrated Protein Science and Adolf-Butenandt Institute[2], Institut Curie[3], Universidad San Sebastián[4].
Much progress has been made concerning histone function in the nucleus; however, following their synthesis, how their marking and subcellular trafficking are regulated remains to be explored. To gain an insight into these issues, we focused on soluble histones and analyzed endogenous and tagged H3 histones in parallel. We distinguished six complexes that we could place to account for maturation events occurring on histones H3 and H4 from their synthesis onward. In each complex, a different set of chaperones is involved, and we found specific post-translational modifications. Interestingly, we revealed that histones H3 and H4 are transiently poly(ADP-ribosylated). The impact of these marks in histone metabolism proved to be important as we found that acetylation of lysines 5 and 12 on histone H4 stimulated its nuclear translocation. Furthermore, we showed that, depending on particular histone H3 modifications, the balance in the presence of the different translocation complexes changes. Therefore, our results enabled us to propose a regulatory means of these marks for controlling cytoplasmic/nuclear shuttling and the establishment of early modification patterns.Acknowledgements: FONDECYT 1090270, Basal Project PFB16, the Iniciativa Científica Milenio-Millenium Institute of Fundamental and Applied Biology (MIFAB) and Institut Curie/CNRS.

114.Transposition of IS*711*, generate a genetic polymorphism in two *Brucella abortus* Chilean strains. Mancilla, M.[1,2], **Ulloa Igor, M.**[1], Lopez-Goñi, I.[2], Moriyon, I.[2], Zarraga, A.M.[1]. Universidad Austral de Chile[1], Universidad de Navarra, Spain[2].
Brucellosis is a zoonosis caused by *Brucella*, a highly homogeneous group of bacteria. The insertion sequence IS*711* is characteristic of these genus, and is present in variable numbers and positions, always constant within a given species. Field isolates of *B. abortus* typically carry seven IS*711* copies. We found two *B. abortus* strains isolated from milk and aborted fetuses that carried two additional IS711 : one in an intergenic region near to the 3' end of a putative lactate permease gene and the other interrupting the sequence of a marR transcriptional regulator gene. Sequence analyses revealed that the new copies probably resulted from the transposition of a single IS*711* copy. The IS*711* transposition has only been shown in vitro and only for *B. ovis* and *B. pinnipedialis*, two species that carrying a high number of IS*711* copies, but never in *B. abortus* specie. Our results show that the replicative transposition of IS*711* can occur under field conditions. This suggests that the transposition is an active mechanism that contributes to intra-species genetic polymorphism and genetic diversity in *B. abortus*.Further work is needed to elucidate if changes promoted by IS transposition are associated with virulence changes in this pathogen.FUNDED BY: FONDOSAG C5-100-10-23, FONDEF D02I-1111, DID-UACh D-2005-17and FICR-EQU18 .

115.Differential recruitment of p160/SRC and DRIP/TRAP co-activator complexes during vitamin D-dependent transcription of target genes in osteoblastic cells. 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 principally occurs through modulation of gene transcription. Binding of vitamin D to the vitamin D receptor (VDR) induces conformational changes in its C-terminal domain establishing competency for interaction with co-activators of the p160/SRC family or the DRIP/TRAP/Mediator complex. It has been established that these two types of co-activator complexes bind to specific target genes in a cyclical, sequential and mutually exclusive manner [e.g. 24-hydroxylase (24(OH)ase)] or alternatively, there is a gradual and preferential association of one of these type of co-activators [e.g. osteocalcin (OC)]. Here we study the contribution of p160/SRC-1 and DRIP205 during the vitamin D-dependent transcriptional response using the model genes 24(OH)ase and OC in osteoblastic cells. By combining chromatin immunoprecipitation (ChIP) and siRNA, we determine that while a decrease in DRIP205 expression reduces vitamin D-mediated transcriptional stimulation of the OC gene transcription, it has only a minor effect on the vitamin D-enhanced 24(OH)ase expression. In contrast, the absence of SRC-1 does not prevent vitamin D-dependent increase in OC gene transcription and reduces responsiveness of the 24(OH)ase gene to the hormone. In addition, we find that both promoters show different patterns of vitamin D-mediated histone acetylation. We propose that the mechanism of co-activator recruitment may reflect the nature of the regulatory elements bound by cognate factors at each target gene promoter and the ability of VDR to preferentially interact with each type of co-activator under specific nuclear environments. CONICYT fellow, FONDECYT1095075, FONDAP15090007.

116.Identification and purification of a histone H3K9 methyltransferase complex from HeLa cytosolic extracts. Díaz Celis, C.[1], Ugalde, V.[1], Dent, S.[2], Almouzni, G.[3], **Loyola, A.**[1,4]. Fundación Ciencia para la Vida[1], UT M.D. Anderson Cancer Center, Texas, USA[2], Institut Curie, Paris, France[3], Universidad San Sebastián[4].
DNA in eukaryotes is compacted into chromatin, of which the basic unit, the nucleosome, is composed of four different histones wrapped by DNA. Chromatin participates in the regulation of gene expression through several mechanisms, including histone variants and histone post-translational modifications. In contrast to the variety of modifications present on histones associated with chromatin (nucleosomal histones), the non-nucleosomal histones H3 and H4, the ones that have not been yet incorporated to the DNA, are poorly modified. Interestingly, the only methylated residue on histone H3 is the lysine 9, which is monomethylated (H3K9me1). Moreover, the H3K9 methylation pattern is different between the non-nucleosomal H3 variants H3.1 and H3.3, with enrichment on the variant H3.1. H3K9me1 promotes the establishment of the H3K9me3 pattern, a characteristic mark of heterochromatin. The aim of this work is to investigate how methylation of non-nucleosomal H3K9 is established. Towards this goal, we performed knock-down experiments of the enzyme SetDB1 in HeLa cells and observed reduced levels of both H3K9 methyltransferase activity and methylation of histone H3K9. We then purified the H3K9 methyltransferase activity from HeLa cytosolic extracts by several chromatographic steps. We monitored the fractions derived from each purification steps by *in vitro* methyltransferase activity and western blot for SetDB1. Consistently, the H3K9 methyltransferase activity and SetDB1 protein co-purified during the purification steps. Our results suggest that the enzyme SetDB1 is the main cytosolic H3K9 methyltransferase. Acknowledgment: FONDECYT 1090270, Basal Project PFB16, ICM-MIFAB.

117.Contribution of lysine methyltransferases and lysine demethylases to bone-specific transcription during osteoblastic differentiation. Rojas Moreno, A.[1], Henriquez, B.[1], Allende, M.[2], Montecino, M.[1]. FONDAP Center for Genome Regulation, Faculty of Biological Sciences, Universidad Andres Bello [1], Faculty of Sciences, Universidad de Chile[2].

Transcription factor Runx2 is essential for osteoblast lineage commitment as it controls the expression of several key bone-phenotypic genes. Transcription of Runx2 is regulated by two promoters P1 and P2 which control the expression of the isoforms Runx2-II/p57 and Runx2-I/p56, respectively. It has been described that stimulation of pluripotent mesenchymal cells with BMP2 (Bone Morphogenetic Protein 2) induces the expression of the isoform Runx2-II/p57. This expression is accompanied by a chromatin remodeling process at the proximal P1 promoter region and a specific pattern of covalent modifications at histones H3 and H4 associated with this sequence. To begin defining the enzymes responsible for this specific epigenetic pattern and their contribution to Runx2 transcription during osteoblast lineage commitment, we have addressed the expression patterns exhibited by the lysine methylases WDR5 and EZH2 as well as the lysine demethylases NO66 and UTX, in C2C12 cells stimulated to engage an osteoblastic differentiation process by incubation with BMP2. These expression studies were complemented with gene knock down experiments, where WDR5, EZH2, NO66 and UTX proteins were down-regulated using lentivirus-driven specific shRNAs. Our results indicate that these enzymes can contribute significantly to Runx2 transcription activation by establishing an epigenetic environment at the P1 promoter that facilitates binding of the transcription machinery. FONDAP 15090007.

118.CBP, p300 and pCAF are associated to chromosomal break point regions. Hinojosa, M.[1,2,3,4], Stuardo, M.[1,2,3,4], Alarcon, R.[1,2,3,4], Martínez, M.[1,2,3,4], Gutiérrez, S.[1,2,3,4]. Laboratorio de Regulación Transcripcional y Leucemia[1], Departamento de Bioquímica y Biología Molecular, [2], Facultad de Ciencias Biológicas[3], Universidad de Concepción[4].

RUNX1 is a key hematopoietic transcription factor required for definitive hematopoiesis and is a frequent target of leukemia-related chromosomal translocations, one of them is t(8;21) usually found in patient with acute myeloid leukemia. To date all the break points mapped for t(8;21) are localized in three cluster regions (BCRs) in intron 5 of *RUNX1* gene. The mechanisms or factors that contribute to formation of this translocation are still unknown. There are no common sequences among *RUNX1* and its partner gene that suggest involvement of a homologous recombination mechanism, however, several chromatin structural element such as Topoisomerase II and DNase I hypersensitive sites have been found in the BCRs. Furthermore, our lab has previously shown that intrón 5 of the *RUNX1* gene exhibit a differential acetylation pattern in hematopoietic cells characterized for hyperacetylation of histone H3 and H4. In order to identify the Histones Acetylases (HATs) responsible for the acetylation pattern previously described, we performed Chromatin Inmunoprecipitation assays (ChIPs) using antibodies against the HATs CBP, p300 and pCAF in hematopoietic cells (HL60). Our results shown that CBP, p300 and pCAF are differentially associated to specific regions in the intron 5, indeed these regions are localized near or inside of BCR's and colocalize with histone H3 and H4 hyperacetylated regions. Our results suggest that the association of CBP, p300 and pCAF to BCRs can modulate the chromatin structure in genomic regions associated with chromosomal translocations. FONDECYT 1100670.

119.Partial characterization of novel antimicrobial components of the hemolymph of the bivalve *Choromytilus chorus*. Hernández, M.[1], Amthauer, R.[1]. Instituto de Bioquímica y Microbiología, Universidad Austral de Chile[1].

Survival of marine invertebrates exposed to pathogen challenges relay exclusively on their robust innate immune system constituted mainly by hemocytes and antimicrobial peptides. Chilean coasts are extremely rich in invertebrates, however very few species have been partially studied. Considering the potential biotechnological interest of bioactive molecules yet to be discovered in these unexplored species, we focused our study in analyzing the components of the hemolymph of the autochthonous bivalve *Ch. chorus* that display antimicrobial activity *in vitro*. Using classic acid- and solid-phase extractions we obtained from *Ch. chorus* hemolymph a moderately hydrophobic fraction that displayed a potent antimicrobial activity against Gram-positive and -negative bacteria but not against fungi (*Candida albicans* and *Cryptococcus neoformans*). In addition, this fraction exhibited strong permeabilization of *E. coli* cells suggesting the presence of peptide(s) able to disrupt bacterial membranes but conveniently not eukaryotic cell membranes. Interestingly, the most abundant hemolymph protein (pMHC) also displayed antimicrobial activity against Gram-positive and -negative bacteria, suggesting it could correspond to an important component of *Ch. chorus immune system. pMHC is a very high MW protein that* sediments after centrifugation at 125.000 x g for 1 h. PAGE-SDS and 2D electrophoresis analyses revealed that pMHC is composed by identical monomers of 75 kDa. Using molecular exclusion chromatography we showed that the native protein dissociated into its monomers after EDTA treatment and reassociated as a high molecular weight multimer after incubation with $CaCl_2$. Funding DID-UACH 200901.

120.Evaluation of the immunogenicity of *P. salmonis* antigens by ELISA assay. Valenzuela, K.[1], Silva, H.[1], Álvarez, C.[1], Saez, M.[1], Cárcamo, J.G.[1], Yáñez, A.[1]. Universidad Austral de Chile[1].

Piscirickettsia salmonis is a Gram-negative, facultative intracellular bacterial pathogen that causes the salmonid rickettsial septicemia (SRS). Although, *P. salmonis* is sensitive in vitro to many antibiotics, infected salmons respond poorly to this treatment, therefore, it is necessary to develop techniques for disease prevention. The goal of this research was to evaluate the immunogenicity of antigens of *P. salmonis* using an ELISA assay for later development a successful vaccine against SRS. For evaluating the antibody presence in fishes, were developed an indirect and sandwich ELISA for IgM anti-*P. salmonis* detection, we used three different capture antigens to estimate which is the best antigen for antibody finding, the positive sample used was a serum of salmon naturally infected. 100 *Salmo salar* were inoculated for intraperitoneal way with 200 or 20 ug of *P. salmonis* total proteins, the negative controls were 25 fishes inoculated with PBS. After 17 and 24 days post inoculation specific antibodies against *P. salmonis* was detected in sera with both ELISA, however, the indirect ELISA showed more difference between negative and positive control. Fishes inoculated with 200ug of protein had greater immune response than fishes inoculated with 20ug, the negative control serum had not specific IgM anti-*P. salmonis response. Thus, we developed two effective ELISA that evaluate* the antibody response in vaccinated salmon and also as expected *P. salmonis* antigens induce a humoral immune response in *Salmo salar*. Support: INNOVA-CORFO project 07CN13PPT-256.

121.Cellular model for evaluating a potential indicator of immunosuppression in salmonids. Palacios, C.[1], Bethke, J.[1], Guzmán, F.[2], Mercado, L.[1,2]. Grupo de Marcadores Inmunológicos en Organismos Acuáticos. Laboratorio de Genética e Inmunología Molecular (GIM), Instituto de Biología[1], Núcleo Biotecnológico Curauma (NBC). Pontificia Universidad Católica de Valparaíso, Chile[2].

Farmed fish are exposed to stress situations like physical changes in the environment, overcrowding, transport and handling. This results in increased levels of cortisol, first inductor of immunosupression, through its anti-inflammatory effect mediated by the protein Lipocortin. This molecule is capable of inhibiting the action of phospholipase A2, the main enzyme involved in the inflammatory pathway. To evaluate a potential indicator of immunosuppression, a primary cell culture model of head kidney leukocytes of O. mykiss was used. HKL cells are actively involved in the inflammatory response and therefore sensitive to the action of cortisol and capable of expressing Lipocortin at protein level. Cells were stimulated for two hours with concentrations of 150, 300 and 600 ng/ml cortisol. Detection of Lipocortin was initially realized through Immunocytochemistry and later by ELISA, using in both cases polyclonal antibodies, whose epitopes were obtained from the primary structure of the sequence of Lipocortin available for O. mikyss. Detection of Lipocortin was evidenced and its major expression was to 300 and 600 ng/ml cortisol in both ICQ and by ELISA. This proposes HKL of O. mykiss, as a valid in vitro model and Lipocortin as a potential indicator of immunosuppression in response to cortisol. Currently there is not a method to determine the immune-suppress condition in fish, therefore if the relationship between cortisol and lipocortin is evidenced at systemic level, the latter molecule could become a novel indicator for the salmon aquaculture industry.

122.Respiratory syncytial virus detection in cells and clinical samples by using three new monoclonal antibodies. Gomez, R.S.[1], Mora, J.E.[1], Cortes, C.M.[2], Riedel, C.A.[2], Ferres, M.V.[3], Bueno, S.M.[1], Kalergis, A.M.[1,4]. Millenium Institute on Immunology and Immunotherapy, Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas[1], Millenium Institute on Immunology and Immunotherapy, Departamento de Ciencias Biológicas, Facultad de Ciencias Biológicas y Facultad de Medicina Universidad Andrés [2], Centro de Investigaciones Medicas, Facultad de Medicina Pontificia Universidad Católica de Chile[3], Departamento de Reumatología, Facultad de Medicina, Pontificia Universidad Católica de Chile.[4].

Infections by Respiratory Syncytial Virus (RSV) are one of the most important health burdens that affect infants worldwide. RSV identification requires specific techniques and equipment, such as immunofluorescence and/or PCR, which are too costly for some public health systems. Therefore, the implementation of rapid, effective and affordable diagnostic tools to diagnose infection by this pathogen is a priority for public health systems. Nasopharyngeal swabs were obtained from 27 RSV-positive patients, 15 hMPV-positive patients and 6 healthy controls. Clinical samples and RSV-infected cells were analyzed by ELISA, FACS, immunofluorescence and dot-blot assays, using three new monoclonal antibodies (mAbs) directed against RSV N and M2-1 proteins that were developed by our group. The majority of samples from infected patients were correctly diagnosed by the mAbs described here. Ratios for correct sample detection were above 0.9 for the three clones analyzed: anti-N clones 1E9/D1 and 8E4/A7, and anti-M2-1 clone 8A4/G9. Further, no false positives were observed for the samples obtained from patients infected with other respiratory viruses, such as hMPV or from healthy controls. Equivalent data were obtained when the RSV-infected samples were analyzed by flow cytometry, immunofluorescence or dot blot, using our three new mAbs. Our results suggest that the anti-RSV mAbs tested in this study can be considered for the rapid and reliable detection of RSV in infected cells and clinical specimens by means of multiple immunological approaches.Work was supported by FONDECYT Grants 1110397; 1100926, FONDEF D06I1008; Biomedical Research Consortium.

123.Characterization of the expression of the T cell receptor Vβ chains in response to the respiratory syncytial virus. Correa, H.G.[1], Mora, J.E.[1], Kalergis, A.M.[1,2]. Millennium Institute on Immunology and Immunotherapy, Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas[1], Departamento de Reumatología, Facultad de Medicina, Pontificia Universidad Católica de Chile.[2].

The respiratory syncytial virus (RSV) is the major cause of bronchiolitis in infants worldwide. Due to uncharacterized virulence mechanism displayed by RSV, the host immune response usually fails to efficiently clear the virus from infected tissues and no protective immunological memory is established. To better understand the immune the nature of the response triggered by RSV antigens, we have analyzed the type of T cell receptors expressed by the T cells responding to the virus. With this aim, BALB/c mice were immunized with different antigens of RSV, UV-inactivated RSV or recombinant BCG expressing RSV antigens. T cells were analyzed 21 days after immunization by in vitro stimulation with purified RSV antigens and measurement of the expression the different TCR Vβ chains by using flow cytometry and RT-PCR. Our preliminary data show the differential expansion of TCR Vβ in response to RSV antigens, suggesting that come particular TCR molecules are responsible of recognizing the virus. The differential expression of particular Vβ chains by RSV-specific T cells could provide an explanation for the modulation of the immune response by RSV. This knowledge could contribute to the design of improved RSV-specific vaccines. Grant Fondecyt 1110397.

124.Expression of proinflamatory cytokines and activation of immune cells of salmonids with protein fractions of *Piscirikettsia salmonis*. Pontigo, J.P.[1], Silva, H.[1], Oliver, C.[1], Valenzuela, K.[1], Olavarria, V.[1], Romero, A.[1], Yañez, A.[1]. Universidad Austral de Chile[1].

Piscirickettsia salmonis, the causative agent of salmonid rickettsial septicaemia (SRS), provokes high losses in the fish farming in the world. Cytokines as modulators of the immune response have been scarcely studied in fish. Interleukin-1beta and TNF-alpha are pro-inflammatory cytokines mainly produced by cell and has been characterized in bony and cartilaginous fish. IL1-beta and TNF-alpha are widely regulated for IKB-alpha in different species, including few fish. The line cell SHK-1 and purified head kidney macrophages of *O.Mykiss* were stimulated with purified bacterial lysate of *P. salmonis*. After the stimulation activation of macrophages through respiratory burst by chemiluminescence and the gene expression of IL1-beta, TNF-alpha and IKB-alpha were evaluated by real time RT-PCR at different times. The expression of IL-1beta showed a significant increase in macrophages incubated with total bacterial lysate of P.salmonis in early time, the expression of TNF-alpha showed a significant increase to late time, for IKB-alpha decrease of the expression was observed in the cells incubated with *P.salmonis*. Different purified extract showed a significant respiratory burst in all cell of salmonids. The modulation of gene expression of cytokine in purified macrophages by different protein extracts of *P.salmonis* and line cell SHK-1 and respiratory burst generate, suggests a correlation of immune innate response in salmonids. The studies are a contribution in the characterization of PAMPS present in *P. salmonis* as part of its pathogenic mechanism. (INNOVA-07CN13PPT-256).

125.Dexamethasone and monophosphoryl lipid A treatment generates monocyte-derived tolerogenic dendritic cells with a stable semi-mature phenotype in healthy volunteers. García, P.A.[1], Hoyos, L.[1], Pesce, B.[1], Morales, R.[1], Pino-Lagos, K.[1], Catalán, D.[1], Aguillón, J.C.[1]. Immune Regulation and Tolerance Research Group[1].

Introduction: Tolerogenic DCs (TolDCs) are capable of inducing tolerance through antigen presentation with defective co-stimulation or by secreting an anti-inflammatory cytokine profile. TolDCs generate an immunoregulatory environment by controlling the activation of effector T cells, which makes them a promising therapy for autoimmunity. Our goal is to generate monocyte-derived TolDCs (Mo-TolDCs) from healthy donors and RA patients and demonstrate their efficacy *in vitro*. **Methods:** A 5-day protocol was developed for generating Mo-TolDCs from healthy donors using the immunosuppressive drug dexamethasone, and the lipopolysaccharide-derivative monophosphoryl lipid A (MPLA). Cells were cultured in AIM-V medium in the presence of IL-4 and GM-CSF. We evaluated DC phenotype in terms of cellular markers and cytokine profile by flow cytometry and ELISA. Tol-DC regulatory capacity was assessed through allo-stimulatory assays with CFSE labeling. **Results:** Mo-TolDCs show characteristic tolerogenic properties such as reduced costimulatory and maturation molecules, anti-inflammatory cytokine secretion profile and reduced allo-stimulatory capacity, shown in co-cultures with CD4+ T cells. Mo-TolDCs maintain their tolerogenic profile when activated with MPLA, only exhibiting higher expression of migratory marker CCR7. We were also capable of identifying TLR2 as possible TolDC marker, highly expressed only at this DC stage differentiation. **Discussion:** We addressed several issues pertaining TolDC generation, such as shorter obtainment time, stability, identification of specific TolDC markers and functionality, the later which is crucial for validating this protocol for future clinical procedures in RA patients.Support: Fondecyt-1100102 and Millennium Institute on Immunology and Immunotherapy- P09-016-F.

126.Generation of antibodies as markers of cell activation in the adaptive immune system of teleost fish. González, R.[1], Narváez, E.[1], Santana, P.[1], Guzmán, F.[1,2], Imarai, M.[3], Mercado , L.[1,2]. Grupo de Marcadores Inmunológicos en Organismos Acuáticos. Laboratorio de Genética e Inmunología Molecular (GIM), Instituto de Biología.[1], Núcleo Biotecnológico Curauma (NBC). Pontificia Universidad Católica de Valparaíso, Chile.[2], Laboratorio de Inmunología, Facultad de Química y Biología. Universidad de Santiago de Chile. [3].

Currently there is a requirement of immunological tools for the identification and classification of cells of the adaptive immune system of fish, however the availability of antibodies is limited. This work describes the generation of monospecific polyclonal antibodies designed against surface markers of T-lymphocytes (CD4) and activated dendritic cells (CD86). Epitope sequences were identified using bioinformatics tools, the candidate regions were selected using the primary structure of these molecules, and subjected to analysis of hydrophobicity and flexibility parameters. The final decision on the choice of the epitopes was done on the simulated 3D structure of each molecule. The candidate peptides were chemically synthesized on solid phase using Fmoc strategy and their molecular weights were confirmed by MALDI-TOF. Monospecific polyclonal antibodies were purified by immunoaffinity using the immobilized epitope on Sepharose 4B CNBr, and the positive immunorecognition was established by ELISA. In addition to surface markers, in the laboratory are antibodies against different interleukins, which may improve the identification of the studied cell types. The cell samples are being standardized from primary cultures of head kidney leukocytes and spleen. Actually the experiments with ELISA and flow cytometry are being realized. Phenotypic characterization of leukocyte cell populations, is a contribution with future projections in aquaculture. The evaluation of vaccines or immunomodulators could be established through indicators that show the activation of the adaptive immune system of fish.

127.Altered gene expression of PHF11-altered Impaired lymphocyte activation and proliferation during atopic dermatitis in humans. Karen Bohmwald[1], Alexis M. Kalergis[1,3] and Arturo J. Borzutzky[1,2] [1]Millennium Institute of Immunology and Immunotherapy. Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas. Pontificia Universidad Católica de Chile. [2]Unidad de Inmunología y Alergia, División de Pediatría, Facultad de Medicina. Pontificia Universidad Católica de Chile.[3]Departamento de Reumatología, Facultad de Medicina. Pontificia Universidad Católica de Chile.

Atopic dermatitis (AD) is a chronic inflammatory condition of skin characterized by immunological hyperresponsiveness to allergens, which is generally considered to be due to Th2-driven immunity and B cell differentiation into IgE producing cells. Severe AD patients have higher IgE levels and more frequent skin infections and show defects on T cells and Th1 immunity. However, disease pathogenesis of AD is still unclear. Genomic studies have found a susceptibility locus for atopy on chromosome 13q14. Further studies have identified that single nucleotide polymorphisms of the plant homeodomain zinc finger protein 11 (*PHF11*) gene in this locus are associated with childhood atopic dermatitis and elevated IgE. PHF11 is strongly expressed in T cells, with preferential expression in Th1 cells as compared to Th2 cells. Moreover, PHF11 has been shown to be relevant for T cell activation and survival. We observed that functional assays of T cell activation and proliferation using peripheral blood mononuclear cells (PBMCs) from patients with severe atopic dermatitis and extremely elevated IgE show deficiencies in lymphocyte activation and proliferation stimulated with mitogens compared with non-atopic control PBMCs. In addition, altered expression of PHF11 mRNA extracted from PBMCs of severe atopic dermatitis patients was observed. These data suggest that T cell dysfunction is associated with severe AD, which may be related to abnormal PHF11 expression. This work was supported by grants from Millennium Nucleus on Immunology and Immunotherapy P04/030-F.

128. Respiratory syncytial virus infection in the central nervous system. Espinoza J:A[1,2], Céspedes P.F[1], Cortés C.M[1,3], Gómez R.S.[3], Riedel C.[1,3] and Kalergis A.M. Kalergis[1,4,*]. [1]Millennium Institute on Immunology and Immunotherapy. Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas. Pontificia Universidad Católica de Chile. [2]Pontificia Universidad Católica de Valparaíso, [3]Facultad de Ciencias Biológicas, U. Andrés Bello. [4]Departamento de Reumatología, Facultad de Medicina. Pontificia Universidad Católica de Chile.

Respiratory syncytial virus (RSV) is the leading cause of bronchiolitis and pneumonia in children, the elderly and immune-compromised individuals. Recently, RSV extra-pulmonary infection, specifically at central nervous system (CNS) has increased the interest relative to viral neurotropism and the generation of CNS disorders as a consequence of infection. Neurologic symptomatology resulting from acute encephalopathy is the second extra-pulmonary manifestation associated to RSV respiratory disease, being characterized by central apneas, seizures, hydrocephaly, strabismus and abnormalities on cerebrospinal fluid composition. Whether these CNS disorders result from either RSV replication or immune system reactivity at nervous tissue is unknown still. To characterize RSV entry, localization and spreading at the CNS would provide insights on virus neuropathology. Using a mouse model for RSV infection, viral proteins and RNA could be detected in brain tissue by immunofluorescence and RT-PCR, respectively. RSV proteins were shown to co-localize mainly with neurons, and in a lesser extent with astrocytes and endothelia. Additionally, Water Maze cognitive tests suggest a delayed learning process on RSV-infected animals. In conclusion, our data support a co-relation between RSV CNS infection and alterations on normal cognitive processes.

Fondecyt # 1110397 Millennium Institute on Immunology and Immunotherapy (P07/088-F).

129.Evaluation of the role of proteins from the respiratory syncitial virus in the disruption of the immunological synapse between T cells and dendritic cells. Alvarez, C.[1,2], Gómez, R.[1], Kalergis, A.[1,3]. [1] Millennium Institute on Immunology and Immunotherapy, Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.[1], [2] Universidad de Concepción[2], [3] Departamento de Reumatología, Facultad de Medicina, Pontificia Universidad Católica de Chile.[3].
Respiratory syncitial virus (RSV) is the leading cause of severe infections in the lower respiratory tract in infants and young children, causing the collapse of public health systems in winter seasons. RSV infection induces an exacerbated inflammatory immune response characterized by an abundant immune cell infiltration into the airways that leads to lung tissue damage. In our group we have recently shown that RSV is capable to impair T cell immunity by inhibiting the ability of dendritic cells (DCs) to assembly activating immunological synapses (IS) with T cells. However, the molecular mechanisms used by RSV to disrupt the IS between DCs and T cells remain unknown. In this study, we have evaluated through confocal microscopy and flow citometry the role of the N and F RSV-proteins in the inhibition of IS formation by RSV. Specifically we have evaluated the recruitment of these proteins to the IS and their contribution to T cell signaling at the IS interface. Our results suggests that the RSV proteins N and F are involved in the inhibition of IS formation by RSV.

130.Exposure to secreted products from HTLV-I infected cells reduces neurite outgrow in PC12 cells. Pando, M.E.[1], Kettlun, A.M.[1], Ramirez, E.[2,3], Cartier, L.[4], Puente, J.[1], Collados, L.[1], Valenzuela, M.A.[1]. Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile[1], Programa de Virología, Facultad de Medicina, Universidad de Chile [2], Departamento de Virología, ISP[3], Departamento de Ciencias Neurológicas, Facultad de Medicina, Universidad de Chile.[4].
Tropical Spastic Paraparesis or HAM/TSP Human T-lymphotropic virus type-I-Associated Myelopathy/Tropical Spastic Paraparesis) is produced by the HTLV-I retrovirus, being characterized by a progressive distal degeneration of the motoneurons axons of the cortico-spinal tract. The virus in vivo infects mainly T CD4+ lymphocytes but not neurons. The secreted protein Tax seems to cause the pathology. In the present work, using PC12 cells as a model of differentiation to neuronal type induced by NGF, we determined the extracellular effect of secreted products from HTLV-I infected T cells (MT-2 cells). PC12 differentiation was followed in the presence of MT-2 secreted compounds or in the absence (with non infected K-562 cells) using a co-culture system separated by a semipermeable membrane. This treatment did not affect the initial process of extending neurite, whereas the further elongation was developed with a significantly reduced rate. Western blots of cell lysates during the differentiation period did not show changes in the levels of postranslational modifications of tubulin (tyrosynated, unstable microtubule marker and acetylated tubulin, stable microtubule marker) therefore, no correlation with the reduction in neurite elongation rate was found. The culture medium contains Tax protein and higher levels of Sema4D compared with the control co-culture. Previous studies in our Laboratory have shown an interaction between Tax and Sema4D, this last protein is involved in neurite growth decrease. This data allows us to propose that extracellular Tax effects are produced through semaphorin receptors. Financial support: Fondecyt 108 0396.

131.SOC influx and activation of NFAT mediates the IL-2 production and expression induced by delphinidin. Jara, E.[1], Hidalgo, M.A.[1], Hancke, J.L.[1], Villalobos, C.[2], Nuñez, L.[2], Burgos, R.A.[1]. Laboratory of Molecular Pharmacology, Universidad Austral de Chile[1], Instituto de Biología y Genética Molecular, Universidad de Valladolid and Consejo Superior de Investigaciones Científicas, Valladolid, Spain[2]. Sponsored by I. Concha.
Anthocyanidins, especially delphinidin, have gained great popularity due to numerous studies that have indicated that they have a lot of potential benefits to human health. These anthocyanins are widely distributed in fruits and vegetables, especially in berries. Delphinidin has been reported possesses antioxidant properties, anti-inflammatory, antimutagenic, antimicrobial and neuroprotective effects. Despite all these findings, nothing is known about its effects on T cell biology. In this study, we determined that delphinidin increase the interleukin-2 (IL-2) production and expression in T cells via SOC (Store-Operated Calcium) influx. Through of spectrofluorimetric experiments and images analyses, and using BAPTA and EGTA, we observed that delphinidin increased cytosolic-free Ca^{2+} by releasing Ca^{+2} from intracellular store and by increasing Ca^{+2} entry. Next, we tested the efficacy of several putative CRAC inhibitors, BTP2 and gadolinium (Gd^{3+}) on Ca^{2+} entry induced by delphinidin. BTP2 and Gd^{3+} reduced the calcium entry stimulated by delphinidin in a dose dependent manner. Moreover, in immunofluorescence and flow cytometry analyses we demonstrated that delphinidin is able of stimulate the NFAT translocation, while through of bioluminescence assays of NFAT activity in single living Jurkat F6 cells, we observed that delphinidin induces the NFAT transcriptional activity. Additionally, we demonstratred that delphinidin induced the expression and production of IL-2 in Jurkat cells, which was reduced significantly by the pre-incubation of the Jurkat cells with BTP2 and Cyclosporine A (CsA). Supported by CONICYT 21090900 and AT-24100037.

132.Taurine activates recombinant Galphas protein. Fernández, P.[1,2], Moreno, J.[1], Barrientos, C.[1], Riquelme, C.[1], León, D.[1], Rodríguez, F.[1], Silva, R.[1], Leonardi, M.[1], Morín, V.[3], Romo, X.[1]. Departamento de Ciencias Biológicas, Facultad de Ciencias Biológicas, Universidad Andres Bello Sede Concepción[1], Facultad de Ingeniería, Ciencias y Administración, Universidad de la Frontera, Temuco, Chile.[2], Departamento de Bioquimica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción[3].
The excessive alcohol consumption (EtOH) has serious effects on public health. Studies have indicated that Taurine (TAU) and Ethanol (EtOH) act in the same way at the Central Nervous System level, potentiating the glycinergic currents. The enhancement of glycinergic currents mediated by EtOH has been attributed to the action of Gbeta gamma of the Gprotein, and has been found that pharmacological concentrations of Ethanol stimulate GDP/ GTPgamaS exchange in Galphas in brain membranes. Because it is only known the in vitro activation mediated by ethanol on protein Galphas, we want to know if TAU acts in the same way than EtOH. To reach this purpose we simulated the interaction between hGalphas with Ethanol and Taurine using Bioinformatics tools. A three-dimensional structure of hGalphas protein was predicted with Modeller9v8 (PDB codes templates: 1TAG-A y 3AH8-A). The models were validated using Prochek and Anolea. For docking studies was used Autodock4.0. The ligands were Ethanol and Taurine (PDB codes: EOH and TAU respectively). On the other way, the hGalphas protein activation in presence of Taurine was studied using recombinant-purified His6-hGalphas protein and doing kinetic experiments of GTPgamaS-Bodipy binding assay. The interaction sites between hGalphas (bound GDP) and ethanol/TAU were in areas close to the switch II and III region, and close to the GDP binding region. On the other hand, the experimental results show a robust increase in GDP/GTP exchange kinetic in presence of TAU, similarly to what happens with EtOH. FONDECYT11080174, DI-16-10/R.

133.Regulation of insulin-induced calcium response in cultured hypertrophic cardiomyocytes. Gutiérrez. T.[1], Parra, V.[1], Pennanen, C.[1], Troncoso, R.[1], Contreras, A.[1], Roberto, B.[1], Vásquez, C.[1], Lavandero, S.[1]. FONDAP Centre for molecular studies of the Cell, Facultad de Ciencias Químicas y Farmacéuticas / Facultad de Medicina, Universidad de Chile. Santiago 8380492, Chile.[1].

Cardiac hypertrophy is a physiopathological condition characterized by an increase in myocardium size with disruptions in calcium and bioenergetic homeostasis and a characteristic insulin resistant state. Our group has previously shown that insulin induces a Ca^{2+} release through ryanodine and IP_3 intracellular receptors, playing the later a key role in glucose uptake and mainly localized in endoplasmic reticulum (ER) surface. Here we evaluate the relationship between insulin signaling desensitization and calcium signaling in norepinephrine-induced hypertrophic neonatal cardiomyocytes. Cytoplasmic and mitochondrial Ca^{2+} signals triggered by insulin (100 nM) were determined by confocal microscopy. The results show that the insulin-dependent increase in mitochondrial Ca^{2+} was significantly reduced in hypertrophic cardiomyocytes respect to non-hypertrophic cells but without changing the cytoplasmic Ca^{2+} increase induced by the hormone. This diminished response in mitochondrial Ca^{2+} was also maintained after stimulation with histamine, suggesting that this abnormal response is related with a reduced Ca^{2+} transfer between ER and mitochondria. Additionally, we quantify the degree of ER and mitochondria colocalization using organelle-selective dyes and Manders coefficients, which suggest a reduced coupling during 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 calcium uptake and dysfunction experienced by this organelle. TG holds a scholarship from Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile. VP, CP, RT, AC and RB are funded by CONICYT. FONDAP 1501006, FONDECYT 1080436.

134.Secreted products from a cell line with a provirus of HTLV-I inserted produce an increase in CRMP-2 phosphorylation dependent on Cdk5. Reyes, J.[1], Iannuzzi, S.[1], Kettlun, A.M.[1], Collados, L.[1], Ramirez, E.[2,3], Cartier, L.[4], Puente, J.[1], Valenzuela, M.A.[1]. Depto Bioquímica y Biología Molecular. Fac Cs Químicas y Farmacéuticas, Universidad Chile[1], ICBM, Programa de Virología, Fac Medicina, Universidad Chile[2], Departamento de Virología, ISP[3], Depto Cs Neurológicas, Fac Medicina, Universidad Chile.[4].

The study of the differentiation process of PC12 is used as a study model of Spastic Paraparesis associated to HTLV-I infection. The damage produced in the CNS is mainly reduced to a degeneration of the long corticospinal neurons, probably related with a failure in axonal transport. The secreted viral Tax protein has been proposed as the main etiological factor that in an extracellular form produced an increase in kinase activities including Cdk5 (shown in studies on SH-SY5Y cells). CRMP-2 is a critical protein expressed in the nervous system involved in transduction signals related with axon growth. The regulation of this protein is mainly based on the phosphorylation at T555 (by Rho kinase), S522 (Cdk5), and at T509 and T514 (by GSK3beta). In this work the differentiation of PC12 cells with NGF was followed in the presence of secreted proteins from a lymphocyte cell line (MT2) infected with HTLV-I, using as control K562 cells (noninfected cells). These secreted proteins, including the viral Tax protein, reduced the differentiation and increase the phosphorylation of CRMP-2. Of the two phosphorylations studied (at T555 and S522) were determined in PC12 lysates by the western blot analysis using specific antibodies. The results showed a significantly increased level of CRPM-2 pS522, but not changes in pT555. These results suggest that the negative effect of MT2 on PC12 differentiation to a neuronal type could be mediated by an increased in the activity of Cdk5. Financial support: Fondecyt 108 .

135.Regulation of ryanodine receptor degradation in the cardiomyocyte. Torrealba, N.[1], Pedrozo, Z.[1], Sanchez, G.[1,2], Donoso, P.[1,2], Lavandero, S.[1,2]. Centro FONDAP Estudios Moleculares de la Célula Facultad de Ciencias Químicas y Farmacéuticas[1], ICBM Facultad de Medicina Universidad de Chile[2].

Ryanodine receptor type 2 (RyR2) has a key role in the heart, releasing calcium from the sarcoplasmic reticulum to activate myocardial contraction. Therefore, RyR2 level and activity are required for the proper cardiomyocyte function and an adequate balance between its synthesis and degradation is important in calcium homeostasis. Our aim was to determine the involvement of chaperone-mediated autophagy (CMA) on RyR2 degradation. To test this, primary cultured cardiomyocytes were exposed to geldanamycin (GA) to induce CMA. RyR2, alpha-spectrin and LAMP-2A levels were assessed by Western blot. Our results show that GA decreased in 50% RyR2 level as compared to control. Lysosome inhibition with NH_4Cl also completely prevented GA dependent RyR2 degradation. Macroautophagy inhibition with 3-methyladenine did not prevent RyR2 degradation. CMA inhibition using LAMP-2A siRNA completely abolished GA-induced RyR2 degradation. The antioxidant NAC also prevented RyR2 degradation but no increase in LAMP-2A levels was observed. ROS inhibition also induced a decrease in cytosolic fragments of RyR2. No increase in calpain activity was detected using alpha spectrin as specific substrate. However, the role of presenilin on RyR2 degradation was evidenced using the specific inhibitor compound E. Taken together, these data suggest that ROS increase could mediate RyR2 oxidation and presenilins could be implicated in RyR2 intramembrane proteolysis which allowed the release of RyR2 fragments to the cytosol and to induce its degradation through CMA. FONDAP 15010006, FONDECYT 1110257, FONDECYT 3110039.

136.Herp controls Beclin-1 degradation by the Ubiquitin-Proteasome System. Gatica, D.[1], Quiroga, C.[1], Paredes, F.[1], Pedrozo, Z.[1], Lavandero, S.[1]. Centro FONDAP Estudios Moleculares de la Célula, Facultad Ciencias Químicas y Farmacéuticas y Facultad de Medicina, Universidad de Chile[1].

Herp is an endoplasmic reticulum (ER) transmembrane protein linked to ER associated protein degradation (ERAD). Herp stimulates Lys48 ubiquitination of certain protein substrates and their proteasomal degradation through the E3 ubiquitin ligase Synoviolin/Hrd1. Beclin-1/Atg6 is a key component for the initiation and progression of autophagy. It has been shown that Lys63 ubiquitination of Beclin-1 dissociates it from its repressor and activates autophagy. Whether Beclin-1 is degraded by the ubiquitin-proteasome system remains unknown. Our hypothesis was that Herp participates on Beclin-1 Lys48 ubiquitination and degradation. To this end, Herp knock-down (KD) HeLa cells and controls were glucose starved for different times then LC3-I and LC3-II levels were determined by Western blot. Beclin-1 levels were also assessed by Western blot in non-stimulated Herp KD HeLa cells. Beclin-1 was immunoprecipitated and blotted with a specific antibody against Lys48 poly-ubiquitin chains. Reverse immunoprecipitation was also performed as a control. Our results indicate that LC3-II levels increased in starved Herp KD HeLa cells in comparison to controls. In the absence of Herp, Beclin-1 content and its Lys48 poly-ubiquitinated form increased and decreased, respectively. Collectively, these evidences suggest that Herp may act as a negative regulator of autophagy by reducing Beclin-1 protein levels through Lys48 poly-ubiquitination and the 26S proteasome. FONDAP 15010006 & FONDECYT 3110039. PF holds a PhD fellowship from CONICYT.

137.ATP is released through hemichannels by Thy-1 - αvβ3 Integrin binding, and upon P2X7 receptor activation triggers migration of astrocytes. Alvarez, A.[1], Kong, M.[1], Quest, A.[1], Leyton, L.[1]. Laboratorio de Comunicaciones Celulares, Centro de Estudios Moleculares de la Célula (CEMC), Facultad de Medicina, Universidad de Chile[1].

Thy-1 is a neuronal glycoprotein ligand of the astrocyte receptors αvb3 Integrin and Syndecan-4. These interactions trigger ATP release and activation of P2X7 receptors (P2X7R), which promote astrocyte adhesion. However, how ATP is released in Thy-1-stimulated astrocytes is still unknown. Furthermore, whether Thy-1 interaction with both receptors is necessary for ATP release remains unresolved. On the other hand, we have evidence that Thy-1 also induces astrocyte migration, but whether ATP release and activation of P2X7R are required for astrocyte migration has not been tested. To address these issues, ATP was measured in astrocyte (rat DITNC-1 cell line) supernatants after different treatments using the Cell Titer Glo kit. A wound-healing assay was employed to evaluate cell migration. Cells were stimulated with Thy-1-Fc wild type or Thy-1 mutated in the integrin and/or heparin-binding sites. We found that ATP release from Thy-1-stimulated astrocytes was prevented by pharmacological inhibition of pannexins, and connexins, but not by an inhibitor of exocytosis. Additionally, only the integrin-binding site was required for ATP release induced by Thy-1, whereas both integrin and heparin-binding sites were necessary for astrocyte migration. Moreover, migration was blocked by a P2X7R inhibitor (BBG). Alternatively, the P2X7R agonist BzATP induced astrocyte migration, which was prevented by BBG. Therefore, upon Thy-1 binding to integrin, ATP is released through hemichannels and activates P2X7R, which induces astrocyte migration in a Syndecan-4-dependent manner. This study describes a novel pharmacological target to regulate the migration induced by Thy-1 in astrocytes.

138.RIC-8B interacts with Gαs and Gαq and is phosphorylated in response to Gas signaling pathway activation. Beyer, A.[1], Pastén, P.[1], Campos, T.[2], Olate, J.[1], Hinrichs, M.V.[1]. Laboratorio de Genética Molecular, Departamento de Bioquímica y Biología Molecular, Facultas de Ciencias Biológicas, Universidad de Concepción[1], Laboratorio de Transucción de Señales y Cáncer, Departamento de Bioquímica y Biología Molecular, Facultas de Ciencias Biológicas, Universidad de Concepción[2].

RIC-8 (Resistant to Inhibitors of Cholinesterase) is a 63kD cytosolic and highly conserved protein, that participates in different cellular processes, like mitosis, differentiation and G-protein signal transduction. In mammals, two paralogs of Ric-8 (RIC-8A and RIC-8B) have been described, which display guanine nucleotide exchange (GEF) activity over different Gα subunits. While RIC-8A is a GEF for Gαq, Gαo and Gαi, RIC-8B acts on Gαs and Gαq. Through two-hybrid and GST-Pulldown interaction assays we demonstrated that hRIC-8B interacts with Gαs through its amino-terminal and carboxi-terminal domain and with Gαq through its carboxi-terminal domain. Previously, 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 Ric-8B phosphorylation. Indeed, through Western-blot analysis, using an anti serine/threonine/tyrosine-phospho antibody, we observed that Ric-8B was phosphorylated in its amino-terminal domain, when cells were incubated with isoproterenol. Since isoproterenol increases intracellular cAMP levels, we looked into Ric-8B for PKA phosphorylation sites. Through a bioinformatic analysis we identified Serine 37 as the putative phosphorylation site, because it presented the highest score and is located in the amino-terminal region of RIC-8B. Mutational analysis of Serine 37 to Alanine will demonstrate if this phosphorylation site is participating in Ric-8 membrane translocation. FONDECYT 1090150.

139.Identification of nucleotide-sugar transporters in grapevines (*Vitis vinifera* L.) Utz, D.[1], Handford, M.[1]. Laboratorio de Biología Molecular Vegetal, Departamento de Biología, Facultad de Ciencias, Universidad de Chile[1].

All plant cells are surrounded by the cell wall, mainly composed of cellulose and non-cellulosic polysaccharides. Cellulose synthesis occurs at the plasma membrane, whereas the non-cellulosic polysaccharides are synthesised in the Golgi apparatus. In this organelle, the glycosylation reactions are catalysed by glycosyltransferases, that recognise specific nucleotide-sugars (NDP-sugar) and transfer the sugar molecule to glycan acceptors. Most nucleotide-sugars, are synthesised in the cytosol and their mechanism of entry into the Golgi lumen is via nucleotide-sugar transporters (NSTs). In Arabidopsis thaliana, the GONST1-5 family of NSTs specific for GDP-sugars and localised in the Golgi. In grapevine (Vitis vinifera L.), it has been determined that the non-cellulosic polysaccharides contain sugars derived from GDP-sugars. To determine the conservation of the mechanism involved in the synthesis of non-cellulosic polysaccharides, the grapevine genome was analysed bioinformatically for the presence of GONST orthologues. Two sequences with ? 78% identity at the amino acid level to GONST proteins were identified, both of which possess the molecular characteristics of NSTs of GDP-sugars. We have called these orthologues VvGONST-A and VvGONST-B. The Cloning of both NSTs was performed successfully and experiments are underway to determine their expression pattern in different grape organs. Future studies will focus on determining the cellular localisation and the substrate specificity of both transporters. Funding: CONICYT Doctorate Scholarship 21090418.

140.Role of glutaredoxin GRXS13 in cold acclimatization process in Arabidopsis. Laporte, D.[1], Salinas, J.[2], Holuigue, L.[1]. Laboratorio de Biología Molecular Vegetal, Departamento de Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile[1], Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Cientificas (CIB-CSIC), Madrid, España[2].

Cold acclimatization is a process that occurs in most land plants exposed to different weather conditions. In Arabidopsis it has been reported that the activation of several pathways confers tolerance to cold stress (chilling) through different mechanisms extensively studied.On another hand, chilling stress produces the accumulation of reactive oxygen species (ROS). This ROS can be responsible for reduced tolerance to cold stress if not detoxified, as shown in mutants that accumulate high levels of ROS.We previously identified GRXS13 ?coding for a putative Arabidopsis glutaredoxin- as a stress-inducible gene. We have shown activation of GRXS13 expression under different stress conditions that induce ROS accumulation. Gain and loss of function analyses indicate that GRXS13 plays a role in Arabidopsis defense response by conferring tolerance to oxidative stress. In this study, we report the increased production of superoxide anion in plants that silenced GRXS13 compared to WT, either under basal or stress conditions. GRXS13 is also activated by chilling in Arabidopsis, effect that is mostly detected in leaf tissues. Moreover, silencing GRXS13 reduced cold acclimatization capacity in plants. These results indicate that GRXS13 has a role in protecting Arabidopsis plants from cold stress.Supported by FONDECYT-CONICYT (grant N° N°1100656) and Millennium Nucleus for Plant Functional Genomics (P10-062-F).

141.Identification and Characterization of Pollen-Specific Promoters in *Arabidopsis thaliana*. **Muñoz, D.**[1]. León, G.[1]. Laboratorio de Reproducción y Desarrollo de Plantas[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 10 candidate genes and confirm the microarray data using RT-PCR for five of them. On the other hand, the putative promoter regions (500 to 1,500 bp upstream the start codon) of these genes were analyzed *in silico*, searching for overrepresented cis elements, which were characteristic in the promoter region of pollen-specific genes. Analysis of plants expressing a reporter gene (GFP/GUS) under the transcriptional control of these putative promoters will allow us to identify promoter sequences highly specific for pollen. Funded by FONDECYT Grant 11080037 and UNAB DI-23/10-R.

142.Development of fluorescent *E.coli* by mobile element insertion in the genomic DNA. **Sáez, M.**[1], Valenzuela, K.[1], Yañez, A.[1]. Universidad Austral de Chile[1].
To study the progression of bacterial infection is required a simple method of pathogen identification. For *in vivo* analyzes is priority to develop new strategies to visualize the infection mechanism (adhesion and invasion) of intracellular bacterial in host cells. The objective of this study was to obtain a fluorescent strain of *E. coli* as models of a non intracellular bacterium by develop a stably transformed bacterial with red and green fluorescent protein. In this work we used transposases to insert an expression cassette coding a fluorescent protein and antibiotic resistance genes in the bacterium?s genomic DNA by two different methodologies. In the first approximation we utilized a cassette flanked by inverted repetitions (ME sequences) and with endogenous expression of the T5 transposase conduce the randomly insertion in of the cassette. In the second approximation we utilized a plasmid with the cassette and outside the cassette is coded a transposase under the control of lac operator and lacI gen. The florescence microcopy result showed the production of an red and green *E. Coli*. Following several passage of the fluorescents strain a great stability of the insertions was observed. In conclusion using this methodology we were able to produce a new fluorescent strain of *E. coli*, technology that can be applied to produce fluorescent human and salmon pathogen. This work was supported by INNOVA-CORFO 07CN13PPT-256.

143.Phenotypic characterization of *Arabidopsis* plants with a mild deficiency in succinate dehydrogenase. **Meneses, M.**[1], Fuentes, D.[1], Gómez, M.I.[1], Jordana, X.[1]. Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.[1].
SDH1-1 is the gene encoding the subunit 1 (flavoprotein) of the mitochondrial respiratory complex II (Succinate Dehydrogenase, SDH) in *Arabidopsis thaliana*. We have previously shown that a null mutation of SDH1-1 is gametophytic, i.e. no homozygous mutant plants can be obtained. Interestingly, a mild reduction of SDH activity in heterozygous mutant plants (SDH1-1/sdh1-1) results in better growth and higher CO_2 assimilation rates, which correlate with increased stomatal density and aperture. Furthermore, while no decreases in chlorophyll or protein content were observed, metabolome analysis revealed decreases in key aminoacids, suggesting higher fluxes to N containing macromolecules and higher nitrogen use efficiency to support increased growth.To investigate if these plants actually use N more efficiently, they were grown on media containing differents concentrations of KNO_3. Interestingly, mutants exhibited increased shoot and root growth when grown on low N-containing media. Moreover, transcripts of nitrate transporters (NRT2.1 and NRT1.1) and nitrate reductase (NIA1 and NIA2) showed increased levels in roots of SDH1-1/sdh1-1 plants. ^{15}N-nitrate uptake was also evaluated and mutant plants exhibited higher ^{15}N-uptake than wild type plants. Altogether, our results suggest that plants with a mild deficiency in SDH activity have a higher photosynthetic performance due to improved CO_2 uptake through stomata, and that this increased CO_2 assimilation leads to better nitrogen assimilation to maintain the C/N balance and support increased biosynthesis of macromolecules and growth.Supported by research grant 1100601 from FondecytChile and the MNPFG (P10.062-F), Millenium Scientific Initiative Program.

144.Phytohormones and light affect the expression of the carotenogenic genes psy2 and lcyb1 in Daucus carota: a direct regulation of their promoters? **Fuentes, P.**[1], Stange, C.[1]. Facultad de Ciencias, Universidad de Chile[1].
Carotenoids are isoprenoid compounds synthesized by all photosynthetic organisms. They are essential in plant physiology due to their roles in photosynthesis, photo-protection and hormone biosynthesis. The carotenogenic pathway is well known, although its regulation is not fully understood. To date, it is known that the main regulatory mechanism takes place at the transcriptional level and that phytoene synthase (PSY) and lycopene b-cyclase (LCYB1) are key regulatory points of this pathway. *Daucus carota* (carrot) storage root accumulates high levels of b-carotene, and carotenogenic genes are transcriptionally regulated during development. Here, we show that the expression of *psy2* is principally associated to carotenoid accumulation in a mature carrot root and that *lcyb1* gene presents the highest induction during development of carrot leaves and roots. Promoter fragments of both genes were obtained by means of Genome-Walker. In silico analysis revealed the presence of phytohormones and light response elements (LRE). Both genes are up-regulated by light in young carrot roots developed under photoperiod but not in mature ones. In carrot seedlings treated with phytohormones, *psy2* was induced by ABA and repressed by 2,4-D, while *lcyb1* was slightly induced by ABA and GA3. Two different *lcyb1* promoter fragments that harbor LRE and not GA3 elements were fussed to *gfp* and stably expressed in *N. tabacum*. *gfp* expression was induced by light, which correlates with the LRE and light treatments of carrot roots and repressed by GA3, unlike the expected results. Aknowledgement to Fondecyt 11080066.

145.Functional characterization of pollen-specific genes through the generation of knock-down transgenic plants expressing intron-hairpin RNAs (ihpRNAs). **Lucca, N.**[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. At maturity, pollen grains are composed by three cells: one large vegetative cell and two sperm cells engulfed in the cytoplasm of the vegetative cell. During fertilization, the vegetative cell germinates and produces 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 452 genes that are only expressed during pollen development in Arabidopsis. Insertional mutants (T-DNA or transposons) were identified *in silico* for 390 of them; however, for 62 genes there are not insertional mutants identified so far, despite the large number of mutant lines available (450,000). To functionally characterize these genes, we have generated transgenic plants expressing intron-hairpin RNAs (ihpRNAs) using a pollen specific promoter (LAT52). Analysis of pollen development, germination and tube elongation will be performed to establish the physiological role of these genes, encoding proteins belonging to different molecular categories. Funded by Fondecyt 11080037 and UNAB DI-23/10-R.

146.Participation of an alcohol acyltransferase (*PhpAAT1*) gene in aroma formation during ripening of goldenberry (*Physalis peruviana* L.) fruit. **Zúñiga, R.**[1], Opazo, M.C.[1], Morales-Quintana, L.[1], Gaete, C.[1], Herrera, R.[1], Moya-León, M.A.[1]. Laboratorio de Fisiología Vegetal, Instituto de Biología Vegetal y Biotecnología, Universidad de Talca[1].
Goldenberry or cape gooseberry (*Physalis peruviana* L.) is a climacteric fruit that develops a characteristic aroma during ripening. As the fruit ripens there is a significant increase in ethylene production and in aroma formation. In *P. peruviana* the main aroma-active compounds produced by the fruit are esters. Esters are synthesized through an esterification reaction catalyzed by the enzyme alcohol acyltransferase (AAT) that utilizes alcohols and acyl-CoAs as substrates. A full-length cDNA (*PhpAAT1*) 1,782 bp was recently isolated from *P. peruviana* fruit. The level of *PhpAAT1* transcripts and AAT activity were determined during ripening of goldenberry fruit. The deduced polypeptide sequence 429 amino acids of *PhpAAT1* displayed the characteristic motifs of most BAHD superfamily plant acyltransferases: the active site motif (HXXXD) and the highly conserved motif located towards the C-terminal end (DFGWG). The transcript accumulation pattern provided by qPCR analysis showed that *PhpAAT1* gene was highly expressed in Y and OR stages, and it was undetectable in vegetative tissues. The increase in *PhpAAT1* transcripts was correlated with the increase in AAT activity. An increase in the production of esters was found during ripening of the fruit which is coincident with the increase in transcript accumulation and AAT activity. These results suggest that *PhpAAT1* gene is involved in aroma biosynthesis in goldenberry fruit. Acknowledgements to Anillo (ACT-41) Project. R.Z. thanks the University of Talca for a PhD fellowship. M.C.O. and L.M.-Q. thank to CONICYT for their PhD fellowships.

147.Molecular strategies to study the function of LCYB2 of *Daucus carota* (carrot), a putative lycopene B-cyclase. **Rosas, C.**[1], Stange, C.[1]. Facultad de Ciencias, Universidad de Chile.[1].
In plants, carotenoids are isoprenoid pigments synthesized in plastids and are involved in photosynthesis, photoprotection and abscisic acid synthesis. In addition, B-carotene, the main carotenoid of carrots, is precursor for vitamin A and possesses high antioxidant properties. Lycopene B-cyclase (LCYB), one of the most important enzymes involved in carotenoid biosynthesis, catalyzes the conversion of lycopene into b-carotene. In plants that accumulate high levels of carotenoids, two genes that encode for LCYB have been described. For instance, pepper harbours *lcyb* and *capsorubin-capsanthin synthase (*ccs) genes, where CCS is responsible for the synthesis of the red pigments capsanthin and capsorubin. In *Daucus carota,* two *lcyb* genes have been described (*lcyb1* and *lcyb2* or *ccs*). During development, *lcyb2* is preferably expressed in a mature carrot storage root. Phylogenetic and aminoacidic analysis showed that *lcyb2* gene is linked with pepper *ccs,* however carrot does not synthesize capsanthin or capsorubine, suggesting that LCYB2 should have LCYB instead of CCS activity. Here, we demonstrated that carrot *lcyb2* present LCYB function by means of heterologous complementation in BL-21/DeltaCrtY *E.coli* strains. *In planta* analysis performed by overexpressing *lcyb2* in tobacco (*Nicotiana tabacum*) showed a significant increase of around 2 fold of b-carotene in transgenic lines related to wild-type plants. We also demonstrated that LCYB2 was targeted to chloroplasts by using transient expression of LCYB2-GFP fusion protein in tobacco. Taken together, these results indicate that carrot *lcyb2* codifies for a functional chloroplast-targeted LCYB. Acknowledgement to Fondecyt 11080066.

148.Profiles and patterns of GRXC9 gene expression under biotic and abiotic stress in *Arabidopsis*. **Villarroel, E.**[1], Herrera, A.[1], Holuigue, L.[1]. Laboratorio de Biología Molecular Vegetal, Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Universidad Católica de Chile.[1].
Salicylic acid (SA) is a plant signaling molecule that triggers the activation of essential genes involved in the defense response against different biotic and abiotic stresses. Previously in our laboratory, we identified genes rapidly induced by SA among which there are genes with putative antioxidant function. In this group we found glutaredoxins (GRX), that catalyze the reduction of disulphide bridges or glutathionylated cysteines from proteins, being crucial for proteins protection under oxidative stress conditions. Within these GRXs genes activated by SA, glutaredoxinC9 (*GRXC9*) has the largest induction level. The *GRXC9* expression profile has been studied in SA treatments; however, its expression during plant development and in stressed plants has not been assessed. For this purpose, we measured *GRXC9* mRNA levels by qRT-PCR and analyzed the expression of GUS and eGFP reporter genes controlled by *GRXC9* promoter in transgenic Arabidopsis different tissues and developmental stages. Furthermore, we examined *GRXC9* expression profiles and patterns in response to biotic and abiotic stresses such as inoculation with *Pseudomonas syringae pv. tomato* (AvrRpm1) and treatments with high light, salt (NaCl), UV-B light and methyl viologen. Our preliminary results show that *GRXC9* is induced under different abiotic stress conditions suggesting a putative role of this protein in these conditions. FONDECYT-CONICYT (N?1100656) and MN-PFG (P10-062-F).

149.Analysis of the expression of AtUTr1 and AtUTr3 genes encoding nucleotide sugar transporters in *Arabidopsis thaliana*. **Cisternas, G.**[*]. Moreno. A.[*]. Orellana, A.[1]. FONDAP Centro de Regulación del Genoma. Núcleo Milenio en Biotecnología Celular Vegetal, Centro de Biotecnología Vegetal, Facultad de Ciencias Biológicas, Universidad Andrés Bello[1].
Nucleotide sugar transporters (NSTs) are responsible of the incorporation of nucleotide sugars into organelles. In *Arabidopsis thaliana*, AtUTr1 and AtUTr3 transport UDP-glucose at the endoplasmic reticulum (ER). Several lines of evidence suggest that AtUTr1 and AtUTr3 are involved in the supply of UDP-glucose for the UDP-glucose: glycoprotein glucosyltransferase (UGGT), an enzyme that is part of the ER quality control through the reglucosylation of misfolded glycoproteins. Thus, AtUTr1 and AtUTr3 would supply the substrate for UGGT. *In silico* analysis of the promoter region of these genes reveal a conserved UPR response element, suggesting that they are regulated by UPR. Since AtbZIP60 and AtbZIP28, are the only transcription factors involved in the transcription of UPR-responding genes, we analyzed whether transcripts of AtUTr1 and AtUTr3 were regulated by these transcription factors by qPCR in mutants in these transcription factors. Also, we determined the expression pattern of AtUTr1 and AtUTr3 across the plant development and under ER stress conditions using GUS reporter lines harboring the promoter of AtUTr1 or AtUTr3 transcriptionally fused to the b-glucuronidase gene. The results show that both genes are upregulated by UPR and this upregulation is partially dependent on AtbZIP60 and AtbZIP28. Moreover, both genes have differential spatiotemporal expression patterns. Finally, biochemical assays using the GUS reporter lines, strongly suggest that transcriptional upregulation of these genes during UPR occur at the promoter level. Fundings by FONDAP CRG-15090007; PCB-MN P02-009F; Fondecyt 1070379. AM is supported by Doctoral Scholarship - CONICYT.

150.Improvement of apple tree by genetic transformation to increase sweetness characteristics in the fruit. **Arcos, Y.T.**[1], Medina, C.[1], Handford, M.[2], Arce-Johnson, P.[1]. Departamento de Genética molecular y Microbiología. Facultad de ciencias Biológicas, Pontificie Unversidad Caólica de Chile.[1], Departamento de Biología de la Facultad de Ciencias, Universidad de Chile.[2].
The classical plant breeding can now incorporate biotechnology procedures such as genetic transformation, to obtain improved plants. In apple (Malus domestica) the modification of the organoleptic characteristics of fruit through the expression of the sorbitol deshydrogenase gene (MdSDH) has been proposed. The natural conversion of sorbitol to other sugars is expected to be enzymatically altered in order to increase sweetness in the Fuji Raku-Raku variety. We standardized a protocol for in vitro culture of buds collected in the field and then for shoot regeneration from young leaves using media supplemented with the phytohormones BAP and TDZ. Genetic transformation requires a selection system for which regeneration assays were performed in the presence of antibiotics. In the case of kanamycin, the minimum concentration required to select the shoots is 15 mg / l and 0.5 mg / l for hygromycin Transformation assays with A. tumefaciens have been conducted using pBI121 containing gus reporter gene and subsequently with the constructs of interest, 35: MdSDH. Transverse leaf segments were used as explants, removing the apical end and the petiole. The explants were allowed to stand in cocultivation for 30 minutes and then were cultured in darkness for four weeks. The shoots in regeneration are being evaluated by PCR and B-glucuronidase assays for the presence of transgenes. Transformation assays using constructions carrying the MdSDH under control of fruit specific promoters will be made in the future. Research the project financed by CORFO-Innova 07-CN-13PBD19.

151.Characterization of *Arabidopsis thaliana* sirtuin 1 activity and function. **Torres, E.**[1], Holzmann, C.[1], Montoya, P.[1], Araya-Secchi, R.[2], Lagos, C.F.[3], Pérez-Acle, T.[2], Jordana, X.[1]. Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontifica Universidad Católica de Chile[1], Computational Biology Lab (DLab), Centro de Modelamiento Matemático (CMM), Facultad de Ciencias Físicas y Matemáticas, Universidad de Chile.[2], Departamento de Farmacia, Facultad de Química, Pontificia Universidad Católica de Chile.[3].
Sirtuins are a family of phylogenetically conserved proteins present in organisms across all kingdoms. They possess NAD^+-dependent enzymatic activity, and produce the deacetylation of lysine residues or the ADP-rybosilation of target proteins. Due to using NAD^+ as a cosusbtrate, changes in NAD^+/NADH ratio have been proposed to modulate sirtuin activity, regulating certain processes in response to the metabolic status of the cell. Two putative sirtuin genes are present in *Arabidopsis thaliana*, *SRT1* and *SRT2*. *In silico* analysis shows that both protein products have conserved domains characteristic of this family. We have shown that Arabidopsis SRT1 has nuclear localization and that heterozygous insertional mutants (*SRT1/srt1*) present decreased viability of pollen, ovules and embryos. Now, using purified recombinant SRT1 we demonstrate it possesses NAD+-dependent deacetylase activity *in vitro*. A molecular model of SRT1/ NAD^+ complex was constructed through comparative modeling with human SIRT6 crystal (PDB 3K35) as template. Several known sirtuin inhibitors were docked against the NAD^+ binding site using molecular dynamics aiming to identify potential modulators of SRT1 activity. Therefore, we expect to evaluate the effect of such chemicals on Arabidopsis SRT1, and to determine some kinetic parameters of its enzymatic activity. Our results suggest that SRT1 may be acting as a nuclear deacetylase, possibly on histones, and that its activity is especially important during gametogenesis and early embryonic development. Fondecyt 1100601, Núcleo Milenio en Genómica Funcional de Plantas P10-062-F, Beca de Apoyo a la Tesis Doctoral AT24100188.

152.The effect of water deficit and high temperature stress on the physiology and biochemical responses of *Aloe barbadensis* Miller (Aloe vera). **Salinas, C.**[1], Ramírez, I.[1], Huerta, C.[1], Freire, M.[1], **Cardemil, L.**[1]. Laboratorio de Biología Molecular Vegetal, Departamento de Biología, Facultad de Ciencias, Universidad de Chile.[1].
Aloe vera is a CAM plant, adapted to arid environments and cultivated in the IV Region of Chile. The objective of this work was to investigate if the water and temperature conditions of this region would affect the plant physiology and the quality of the gel which has a commercial value. For this, we determined the water use efficiency (WUE) and gel production of plants under different water regimes and temperatures. We also quantified by semi quantitative RT-PCR the expression of genes encoding proteins associated with stress responses such as the HSP, ubiquitin, and superoxide dismutase, and the accumulation of these proteins by western blot analyses. The presence and concentration of sugars and polysaccharides responsible for the osmotic adjustment (fructans) of the plant and for the economical qualities of the gel (galactoglucomannan) were also evaluated. Our results indicate that Aloe vera increased the WUE under water deficit, due to an efficient osmotic adjustment. The plant showed an increase in gene expression and accumulation of the stress protection proteins. Total sugar increased and analysis of partially methylated alditol acetates by GC-MS of polysaccharides showed that the glycosidic linkages of the fructans and galactoglucomannan changed during drought. Our results indicate that heat stress and drought induce physiological and molecular responses in Aloe vera, changing the composition of the gel which could affect the commercial value of the plant. Supported by MULT 05/30-2 of DI, Universidad de Chile and FONDECYT 1070899 and 7080094.

153.Structural Characterization of LLP Protein (Lectin Like Protein) from *Arabidopsis thaliana* and Expression Pattern Analysis under Biotic Stress. García Mardones, C.[1], Armijo, G.[1], Holuigue, L.[1]. Departamento de Genética Molecular y Microbiología. Facultad de Ciencias Biológicas. Pontificia Universidad Católica de Chile.[1].

LLP was identified as an early-responsive gene under salicylic acid (SA) treatments in *Arabidopsis thaliana*. Its role in the defense response to avirulent strains of *Pseudomonas syringae* is suggested by SA-mediated activation after bacterial inoculation and bacterial proliferation decrease in *LLP*-overexpressor lines. Additionally, LLP protein would be located in the plasma membrane Due to its unknown biological function, we aim to characterize LLP structure and expression patterns in bacterial inoculated tissues, in order to understand its role in biotic stress. For this purpose, we looked for patterns and domains to evaluate functional relations of LLP to the lectin family in Arabidopsis. We found that *LLP* codes for a carbohydrate binding protein from the legume lectin family. Moreover, two putative *N*- glycosylation sites are present in its sequence, suggesting that LLP goes through the secretory pathway. Based on homology modeling we demonstrated that the LLP three-dimensional structure is similar to the legume lectin fold. Interestingly, highly conserved residues from legume lectins involved in carbohydrate-binding are not present in LLP primary structure. Nevertheless, and according to reported amino acid-carbohydrate interactions, non-canonical residues could play this role. Experimentally, we demonstrate the presence of glycans attached to LLP, by enzymatic deglycosylation assays and by using different detergents we also indentified LLP as a peripheral protein. We are currently evaluating the patterns of LLP accumulation during *Pseudomonas syringae* infections, by expressing the LLP-GFP fusion protein under its own promoter. FONDECYT(1100656) and Millennium .

154.Expression and function of Sirtuin 2 in *Arabidopsis thaliana.* Montoya, P.[1], Holzmann, C.[1], Jordana, X.[1]. Facultad de Ciencias Biológicas, Departamento de Genética molecular y microbiología, Pontificia Universidad Católica de Chile. [1].

Sirtuins are a family of proteins conserved in all kingdoms of life. They are involved in different functions such as transcriptional silencing, chromosomal stability, metabolic control, even in longevity. Because sirtuins respond to an increase in NAD^+/NADH ratio, they are key regulators of the metabolic status of the cell. In plants there are two sirtuin like proteins, SRT1 and SRT2. Several splicing variants have been described for the Arabidopsis SRT2 locus. We have confirmed that the SRT2 pre-mRNA is alternatively spliced and that at least 3 encoded proteins are localized into mitochondria. Considering that several differential splicing events occur in the 5' gene region, we designed specific primers in this region to analyze the expression of the splicing variants in different tissues and growing conditions. Interestingly, alternative splicing appears to be regulated in a tissue-specific and/or developmental way. Furthermore, we have obtained two T-DNA null mutants, which have reduced hypocotyl growth in the dark only when deprived of sucrose. These results suggest that different forms of mitochondrial SRT2 could regulate mitochondrial activity and that STR2 is required for proper growth in heterotrophic conditions, for instance for sucrose synthesis from seed storage reserves. Fondecyt 1100601. Núcleo Milenio en Genómica Funcional de Plantas P10-062-F. Beca de Apoyo a la Tesis Doctoral AT24100188.

155.Effect of postharvest calcium chloride and naphthalene acetic acid (NAA) applications on cell wall degradation of Chilean strawberry (*Fragaria chiloensis*) fruit. Vera, P.A.[1], Diaz, M.[1], Arriagada, O.[1], Opazo, M.C.[2], Moya-León, A.[2], Figueroa, C.R.[1,3]. Facultad de Ciencias Forestales, Universidad de Concepción, Casilla 160-C, Concepción, Chile.[1], Instituto de Biología Vegetal y Biotecnología, Universidad de Talca, Casilla 747, Talca, Chile.[2], Centro de Biotecnología, Universidad de Concepción, Casilla 160-C, Concepción, Chile.[3].

Chilean strawberry is a highly perishable fruit. Research has shown that calcium treatment delays fruit deterioration by maintaining cell wall integrity and that NAA represses expression of some ripening-associated genes in strawberry. The objective of this investigation was to reduce cell wall degradation of *F. chiloensis* fruits during storage by $CaCl_2$ and NAA applications. Ripe fruits were dipped in solutions containing $CaCl_2$, NAA, or both combined at 45°C during 15 min. Fruit were stored and samples were taken at 0 days, 5 days at 2°C, and 48 hours after 8 days of cold storage. Fruit quality parameters, changes in cell wall polymers and transcriptional levels of several genes related to cell wall modification were evaluated. No differences in firmness, acidity, and soluble solids content were detected after cold storage between treatments; however weight loss was lower in NAA-treated fruit. Calcium-treated fruit showed a low solubilisation of pectic polymers and high hemicellulose content during cold storage. The combined treatment produced low transcriptional levels of pectate lyase (*PL*) and expansin-2 (*Exp2*) genes during all storage time. In addition, an increase in the expression levels of pectin methylesterase (*PME*) and xyloglucan endotransglycosylase/hydrolase (*XTH1*) genes was observed after cold storage in calcium- and NAA-treated fruits, respectively. In conclusion, calcium plus NAA addition could help the cell wall integrity by reducing expression levels of key genes, like *PL* and *Exp2*, during postharvest of *F. chiloensis* fruits.Work funded by Proyecto FONDECYT Postdoctoral 2010 No. 3100031.

156.Functional analysis of LLP (lectin-like-protein) promoter of *Arabidopsis thaliana* in response to salicylic acid and biotrophic pathogens. Seguel, A.[1], Armijo, G.[1], León, L.[1], Holuigue, M.L.[1]. Laboratorio de Biología Molecular Vegetal. Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.[1].

LLP (*lectin-like-protein*) gene from Arabidopsis codes for a protein with similarity to legume-lectin family that has not been associated to a biological function. This gene was identified in our group as a gene activated by salicylic acid (SA), a plant hormone fundamental in the defense response against different types of stress, particularly biotrophic pathogens. *LLP* is also activated by inoculation with avirulent bacteria Pseudomonas syringae pv. tomato AvrRpm1 and this activation is SA-dependent. *LLP* activation by SA requires the co-activator NPR1 and TGA class II transcription factors (TGA2/5/6). These results guide us to study the *LLP* promoter as a model to understand the activation mechanism of NPR1-dependent genes by SA and pathogens. With the purpose to identify possible promoter elements involved in *LLP* induction, we identified a group of genes that are co-expressed with *LLP* and used a motif sampler analysis to identify over-represented motifs related to plant defense in the upstream sequence of these genes. In parallel, we made genetic constructs using the *LLP* promoter fused to *GUS* and *GFP* as reporter genes for stable transformation in Arabidopsis. The analysis of these transgenic plants indicated that *LLP* regulation occurs at transcriptional level and that *LLP* promoter responds to SA treatment and avirulent pathogen inoculation. Through this approach we try to document the spatio-temporal promoter activity under treatments with different strains of *P. syringae*, as well as to elucidate the promoter elements responsible for *LLP* activation.FONDECYT (1100656), Millennium-Nucleus-PFG (P10-062-F).

157.FNR of *Acidithiobacillus ferrooxidans*: predicted transcriptional regulator for anaerobic growth. **Osorio, U.**[1], Jedlicki, E.[2], Holmes, D.S.[1]. Center for Bioinformatics and Genomic Biology, Fundación Ciencia para la Vida, Santiago, Chile and Depto. de Ciencias Biológicas, Facultad de Ciencias Biológicas, Universidad Andrés Bello, Chile.[1], ICBM, Facultad de Medicina, Universidad de Chile, Santiago, Chile.[2]. Sponsored by O. Orellana..

Acidithiobacillus ferrooxidans is a biomining bacterium able to live in extreme conditions such as low pH, high heavy metal concentrations and low oxygen concentrations. The capacity of *A. ferrooxidans* to grow using alternative electrons carriers in anaerobic conditions has previously been described. However, little is known about additional pathways used in anaerobosis nor about the genes regulating these pathways. In this work, we report the identification and purification of FNR (Fumarate Nitrate Reductase) predicted to be involved in the transcriptional regulation and adaptation of *A. ferrooxidans* in anaerobic conditions. We also present data describing the presence of a cofactor, iron content and oxygen sensibility of FNR and we suggest how these properties could be involved in the function of FNR as a master transcriptional regulator of genes involved in anaerobic growth. Potential DNA binding sites of FNR in promoter regions of genes were predicted, allowing a preliminary model to be built of anaerobic growth in *A. ferrooxidans*. This information could be useful for industrial bioleaching as well as for advancing fundamental insight into this extremophile.Acknowledgments: Fondecyt project 1090451, Mecesup UAB0802.

158.Metabolization of labelled giberellin precursors by Rhizobium bacteroids from soybean nodules. **Méndez, C.**[1], Montanares, M.[1], Baginsky, C.[2], Caru, M.[3], Hedden, P.[4], Rojas, M.C.[1]. Departamento de Química, Facultad de Ciencias, Universidad de Chile[1], Departamento de Producción Agrícola, Facultad de Ciencias Agronómicas, Universidad de Chile[2], Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile[3], Rothamsted Research, UK[4].

Gibberellins (GAs) are diterpene phytohormones synthesized by some rhizobacteria as part of their interaction mechanism with host plants. Particularly GA biosynthesis genes have been described in Bradyrhizobium japonicum, a soybean (Glycine max.) simbiont. In this system two genes codify for the cyclases involved in ent-kaurene biosynthesis, the first commited intermediate of the pathway: ent-copalyl diphosphate synthase (CPS) and ent-kaurene synthase (KS), functionally characterized in E. coli. Besides, three P450 monooxygenase genes that would be involved in oxidative reactions after ent-kaurene form part of the GA biosynthesis gene operon. Upon plant infection the bacteria enters the root cells generating 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. It has been proposed that the microaerophilic environment within the nodule would be essential for the expression of the GA genes. In this work bacteroids were isolated from root nodules of soybean plants and the activity of GA oxidases was assayed in bacteroid suspensions by adding ^{14}C-labelled GA precursors. Several oxidation products were detected as expected for the enzymes of the GA pathway. Bacterial isolates obtained from active nodules were found to correspond to Rhizobium strains and were screened for cps and ks GA biosynthesis genes utilizing the primers described for B. japonicum. Some of the isolates showed amplification bands of the size expected for cps and/ or ks. FONDECYT 1110127.

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